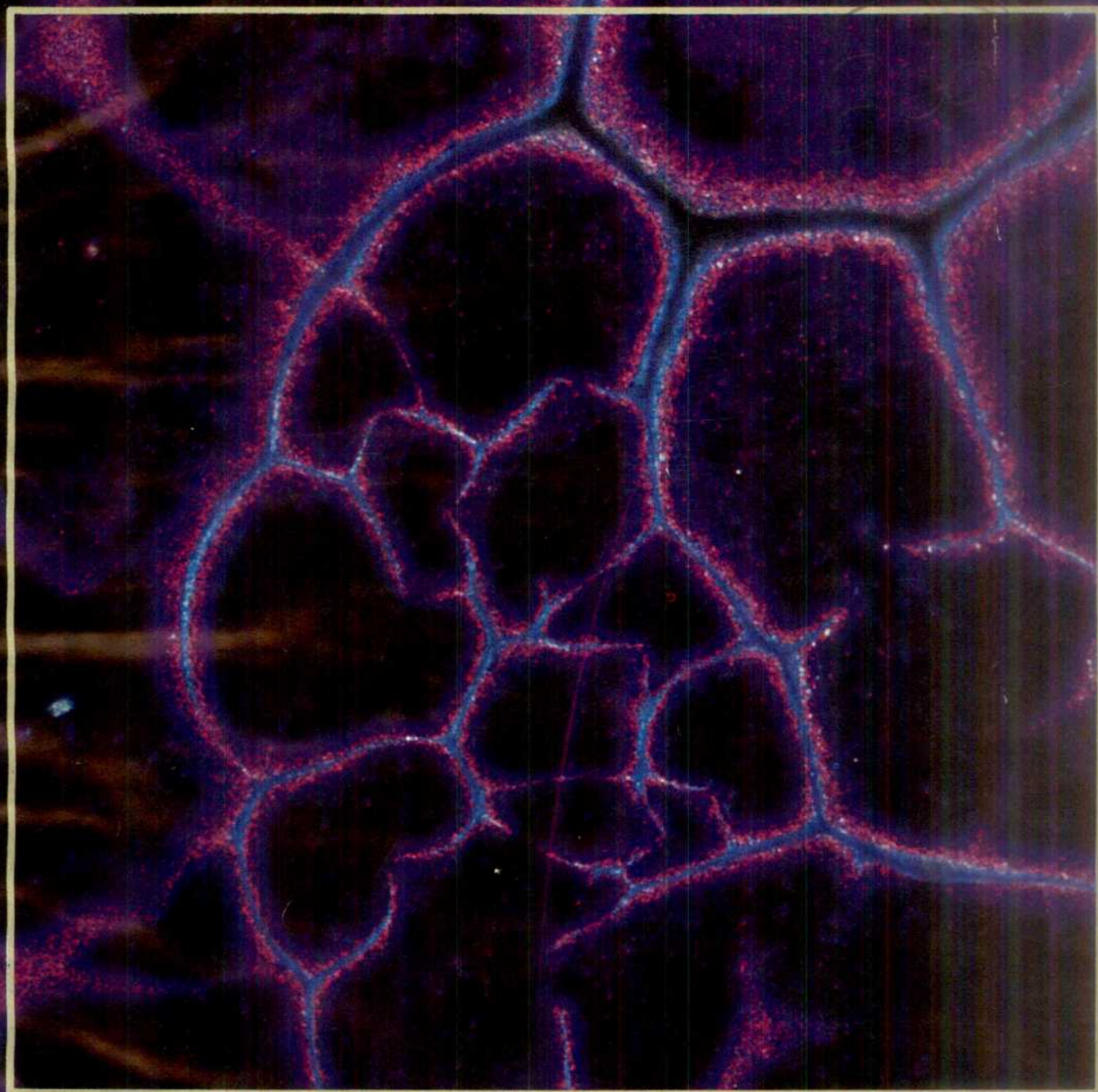


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Shrinkage cracks in montmorillonite-water gel deposited on a glass slide. The filaments, which consist of oriented aggregates of small platelets, will re-expand on contact with water to fill the spaces. But this ability is destroyed by reaction with steam, with possible consequences for the efficiency of clay barriers proposed for nuclear waste repositories. See p.50.

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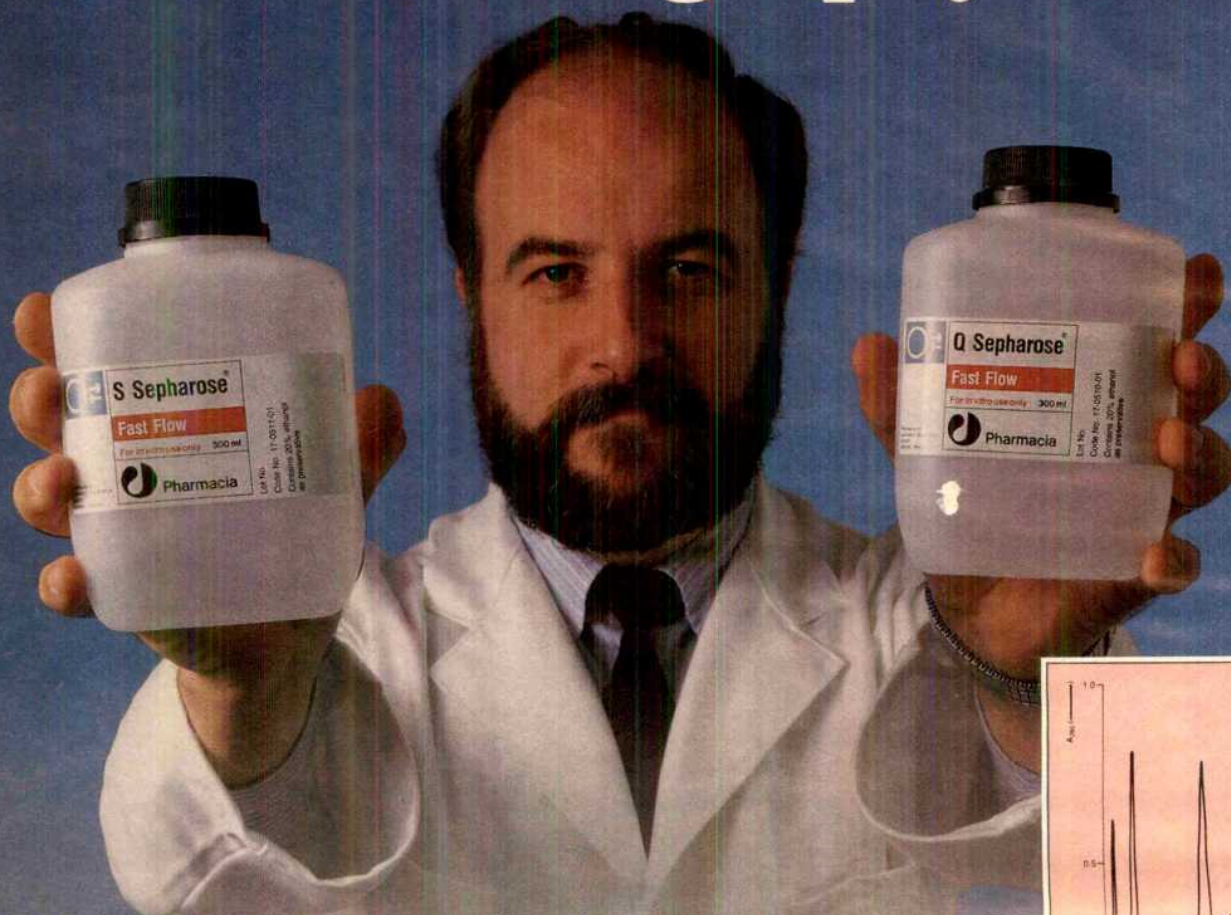
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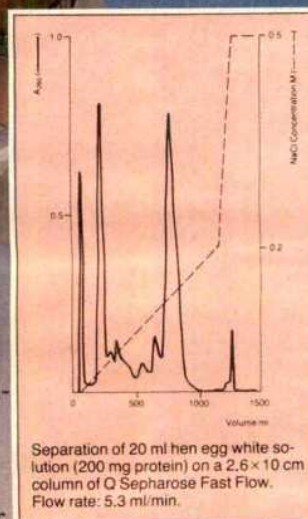
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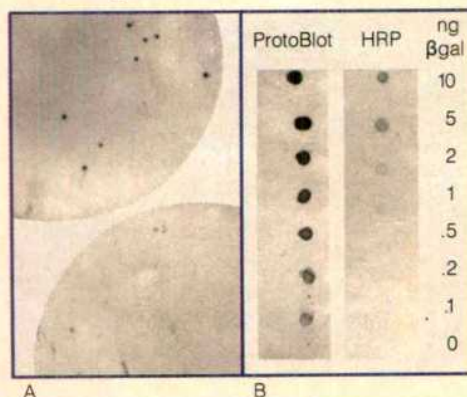
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A. Plaque lift comparison. Approximately 20 recombinant λ gt11 phage containing an ovalbumin cDNA were plated with 10^4 λ gt10 phage on a Y1090 host. After a 3.5 hour incubation at 42°C the plates were overlaid with IPTG soaked nitrocellulose filters and incubated for another 3.5 hours at 37°C. The filters were processed according to recommended protocols with either alkaline phosphatase conjugated second antibodies (ProtoBlot system, upper left) or horseradish peroxidase conjugates (lower right). The primary antibody in both cases was a rabbit anti- β -galactosidase IgG fraction, which detects the ovalbumin/ β -galactosidase fusion protein expressed by the λ gt11 recombinants. Low backgrounds are indicated by the absence of visible λ gt10 plaques.

B. Dot blot comparison. The indicated amounts of purified β -galactosidase were spotted in one microliter volumes on nitrocellulose. The filters were processed under the same conditions as the plaque lifts. Development times in both experiments were 6 minutes for the ProtoBlot system (left lane) and 30 minutes for the HRP system (right lane).

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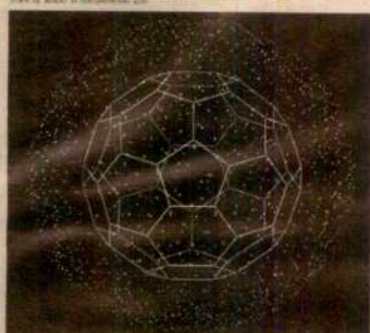


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A computer-generated model of a novel molecule formed by laser irradiation of graphite. The 60-carbon molecule is remarkably stable, with a structure akin to a truncated icosahedron — a polygon with 60 vertices and 32 faces, 12 of which are pentagons and 12 hexagons — a geometrical pattern commonly encountered in the form of a football (soccer, that is). See page 162.

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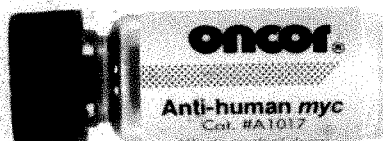
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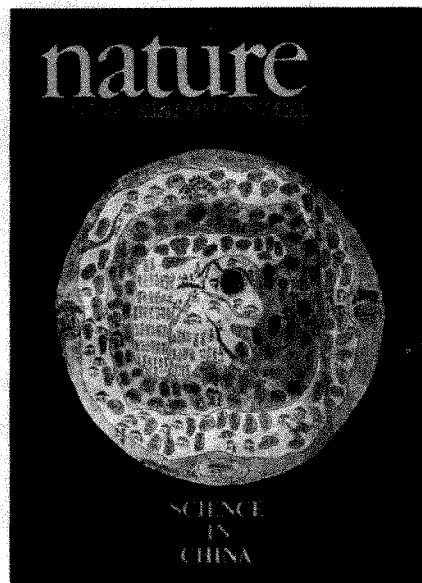
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A 'religious' style of map-making flourished in eighteenth-century China, alongside the more scientific tradition. China is allotted the central place of honour, surrounded by fabulous seas and mythical countries, plus a few real countries including Korea and Japan. Science in twentieth-century China is the subject of this week's supplement, starting on page 205. (Map published circa 1800 in Korea; by permission of the British Library.)

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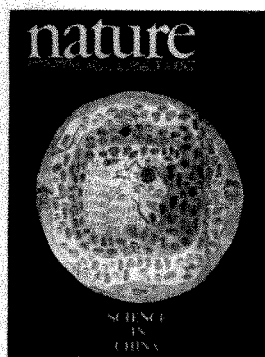
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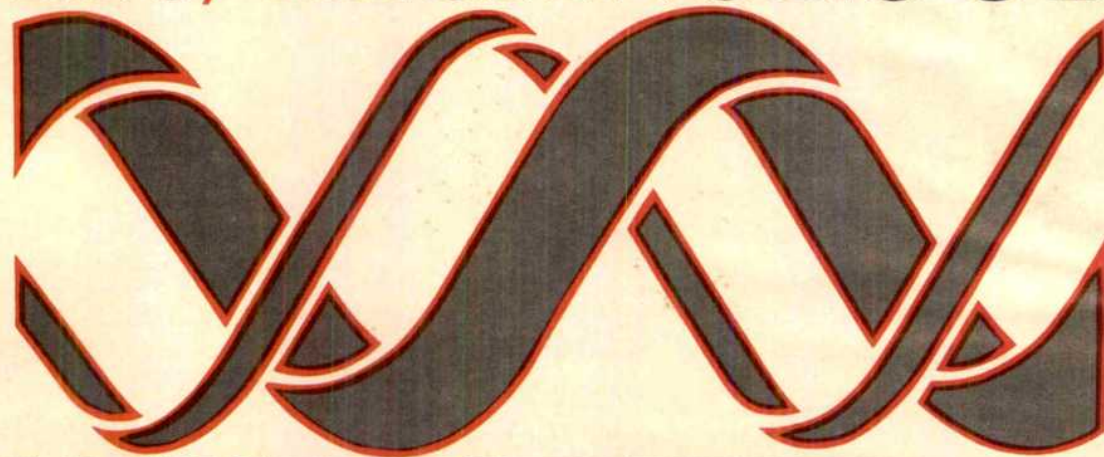
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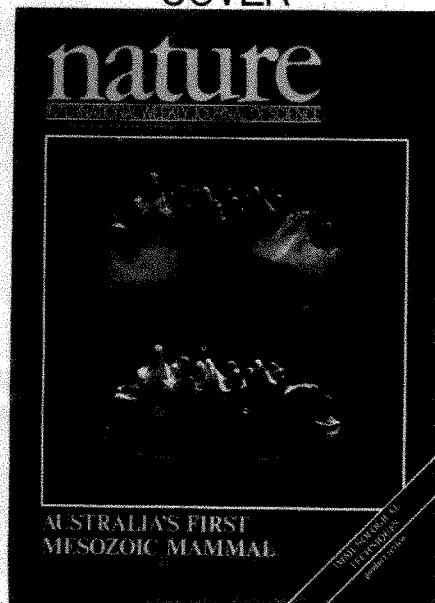
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The opalized remains of Australia's first-known Mesozoic mammal, an Early Cretaceous monotreme from Lightning Ridge, New South Wales. Its structure supports the view that monotremes are closely related in terms of evolution to the other two groups of living mammals — the marsupials and placentals. See page 363 and News and Views p.313. (Photo: John Field.)

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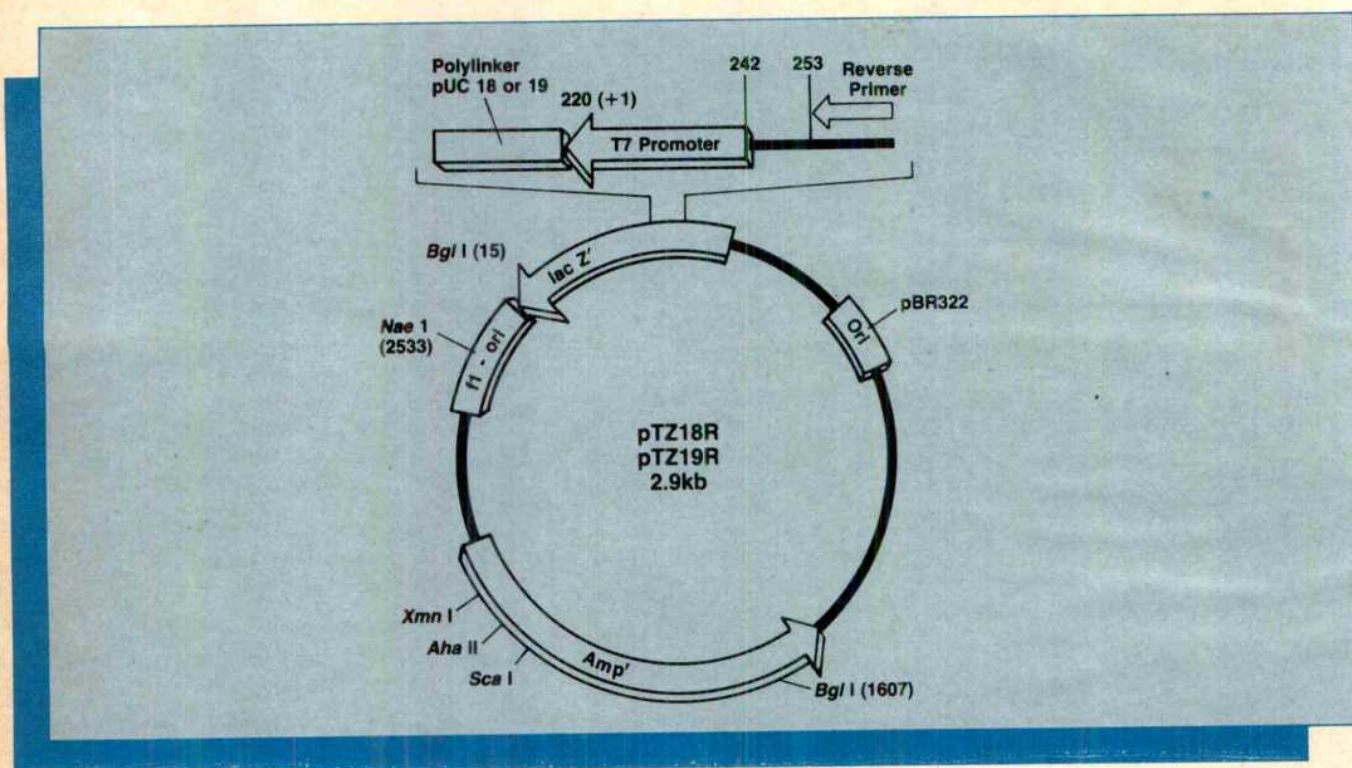
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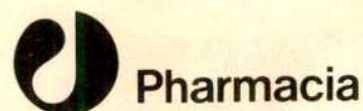
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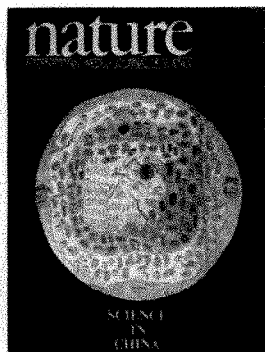
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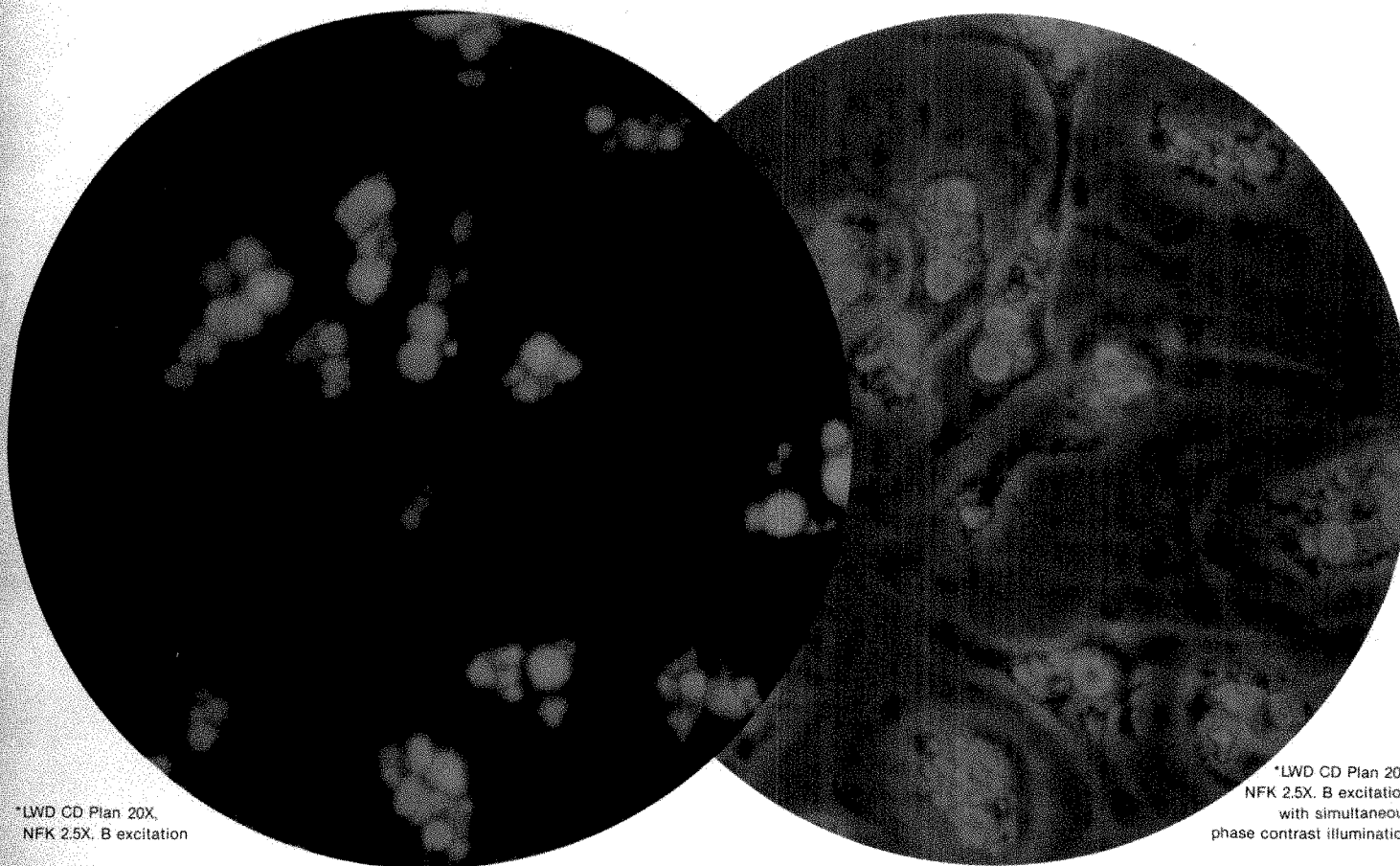
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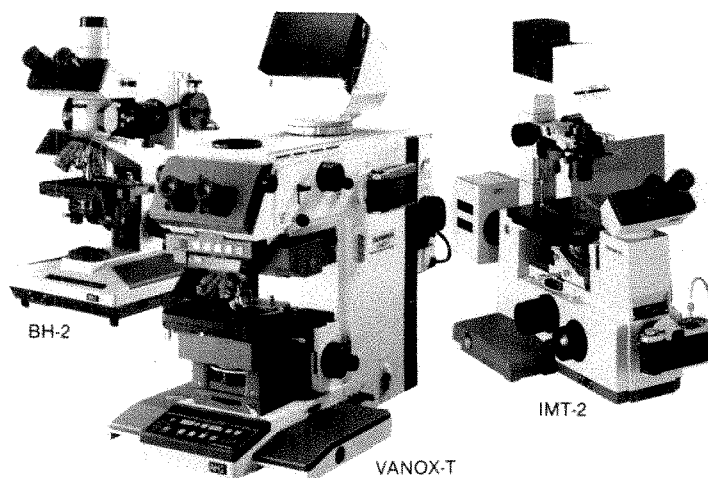
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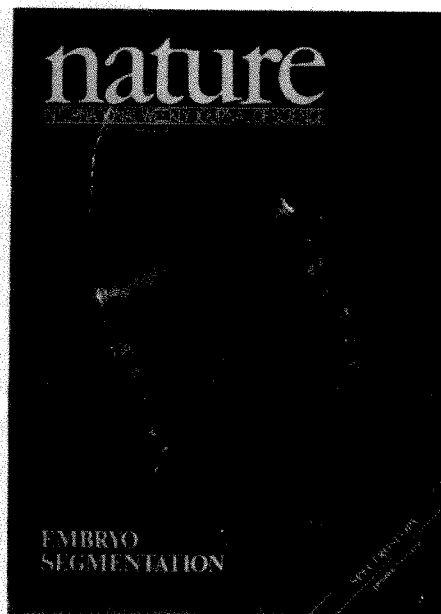
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The developing pattern of expression of the *engrailed* gene in an early *Drosophila* embryo. At later stages the pattern of expression of *engrailed* corresponds to that of morphological segmentation, but the cover photograph shows this is preceded by a stage with a two-segment periodicity. As discussed on page 433 of this issue, this could reflect a fundamental aspect of the pattern-forming process of embryonic segmentation.

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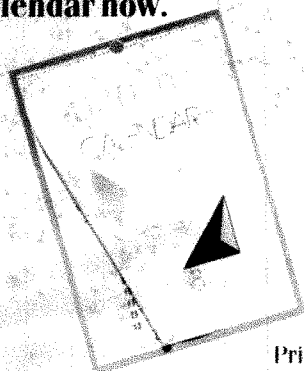
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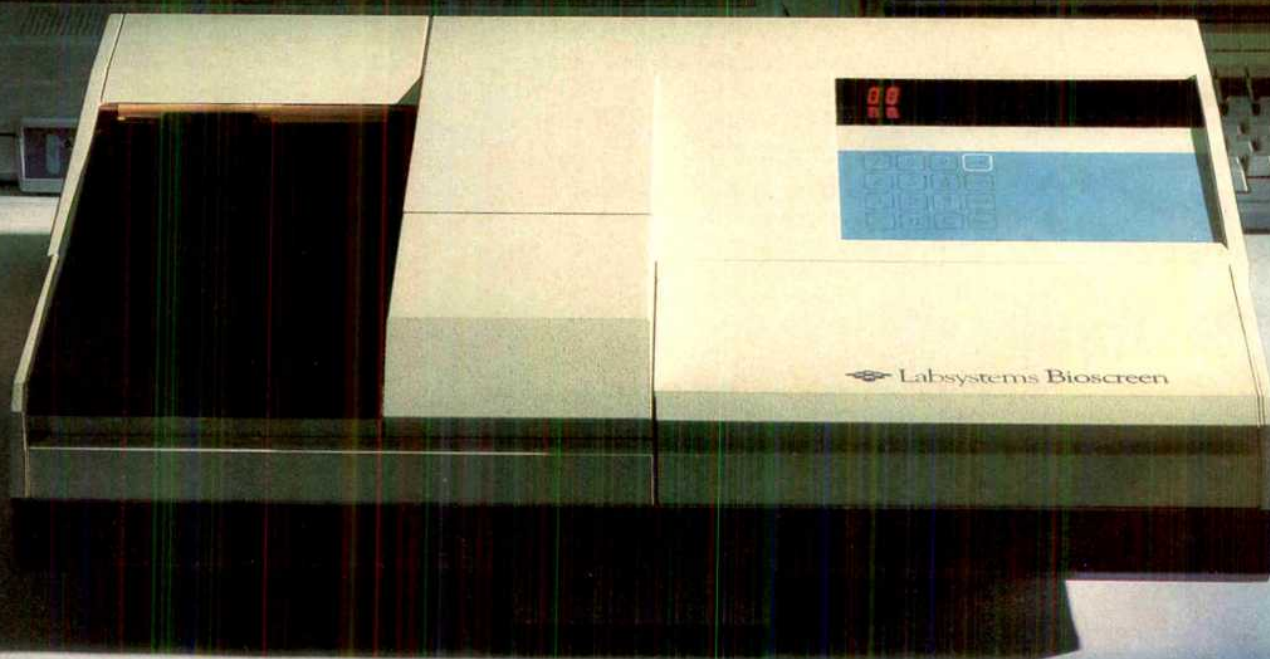
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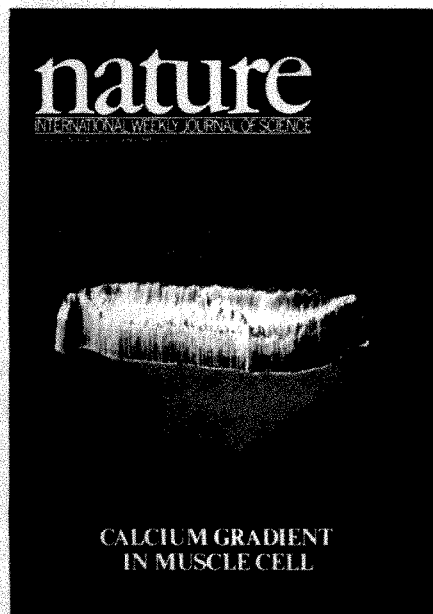
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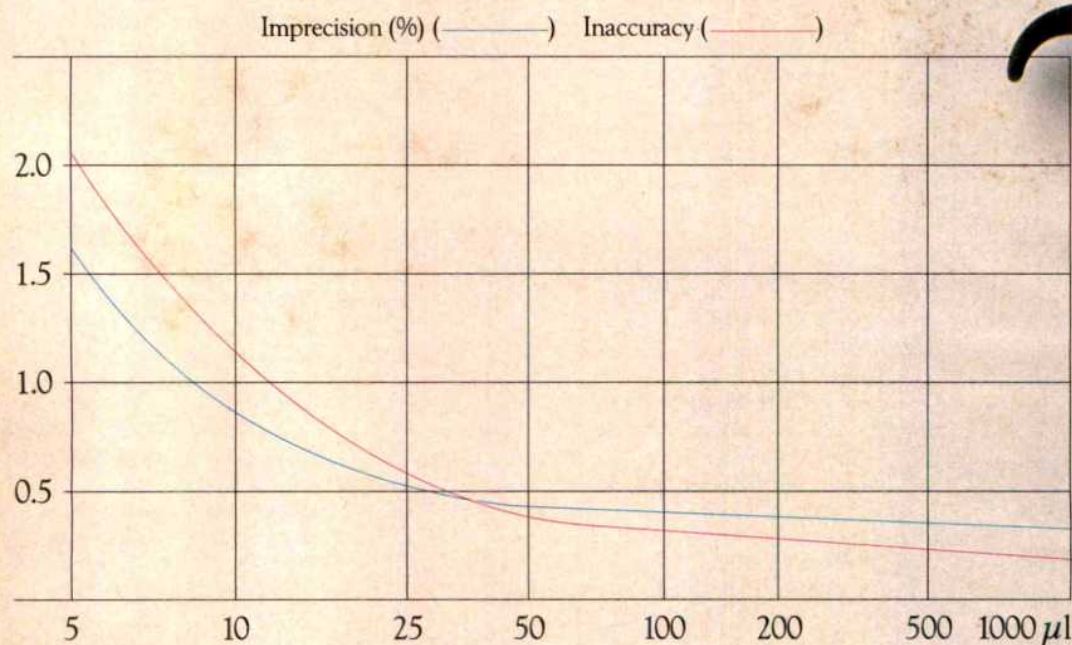
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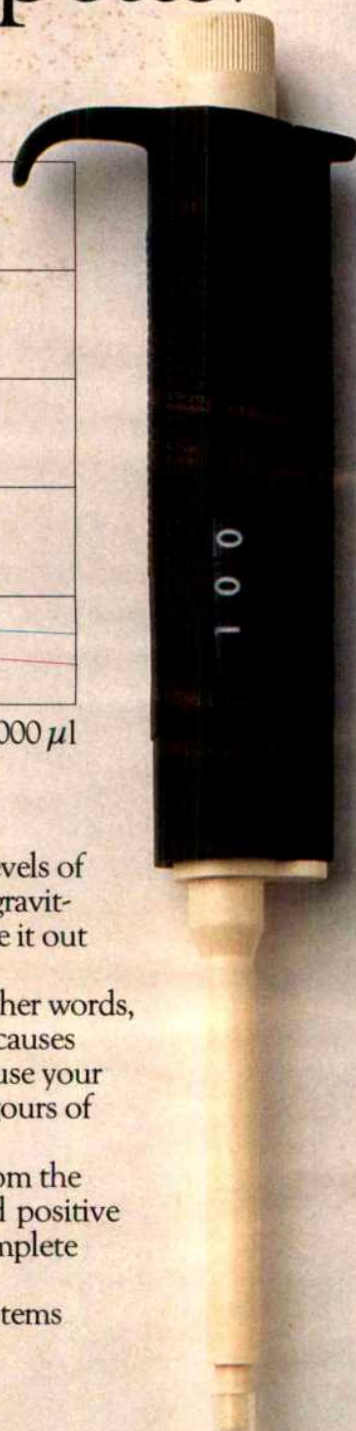
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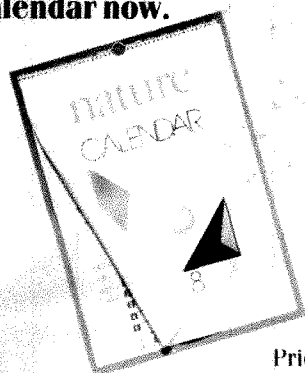
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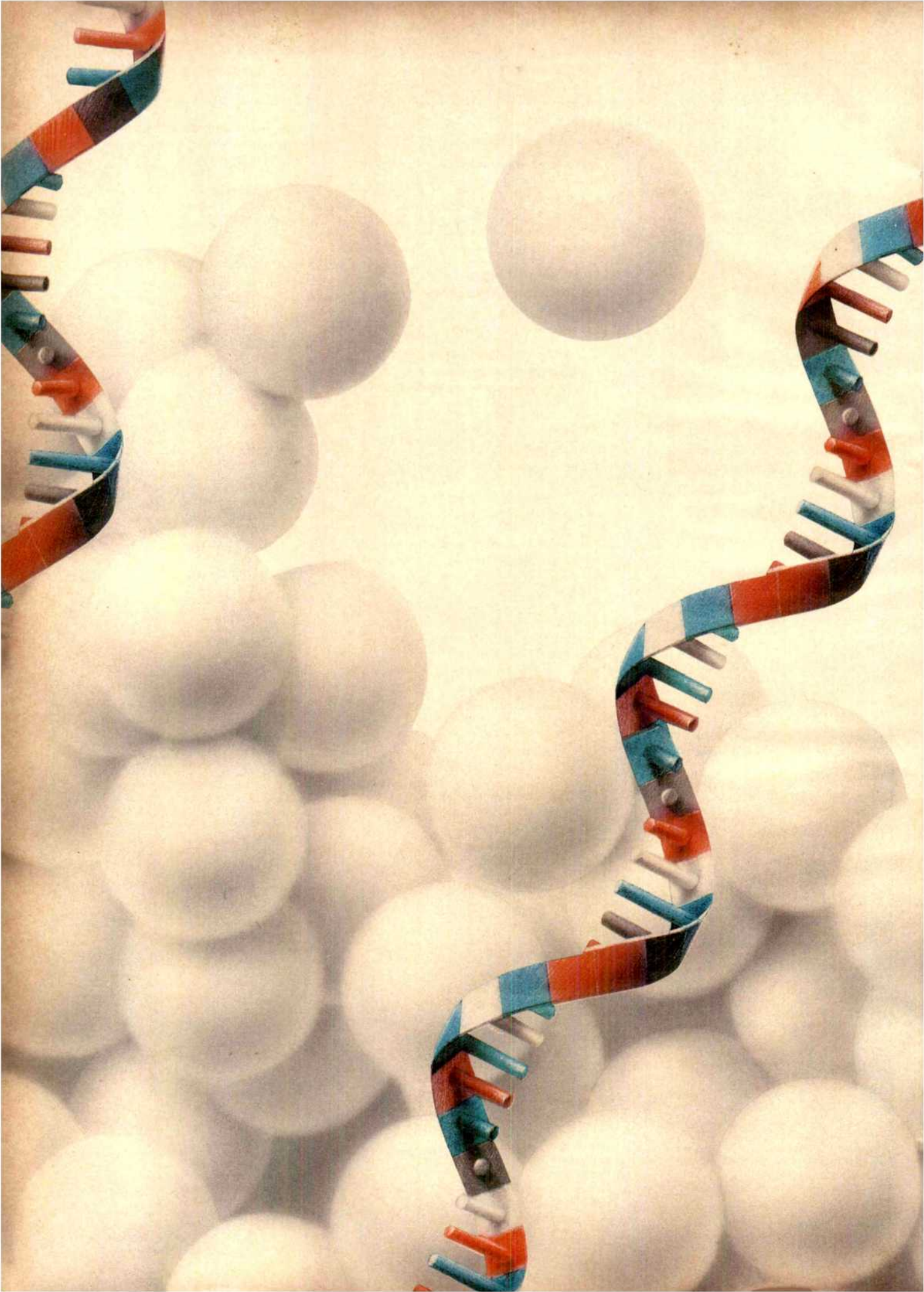
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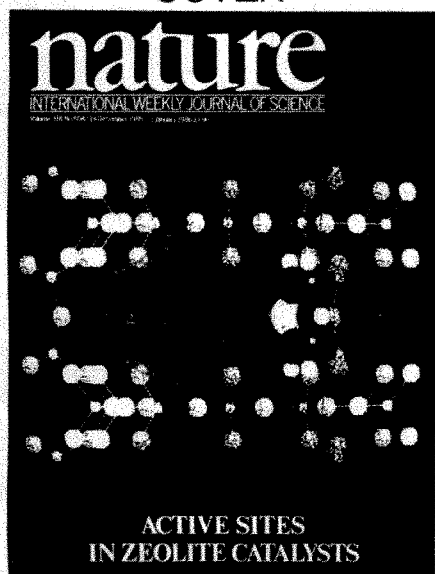
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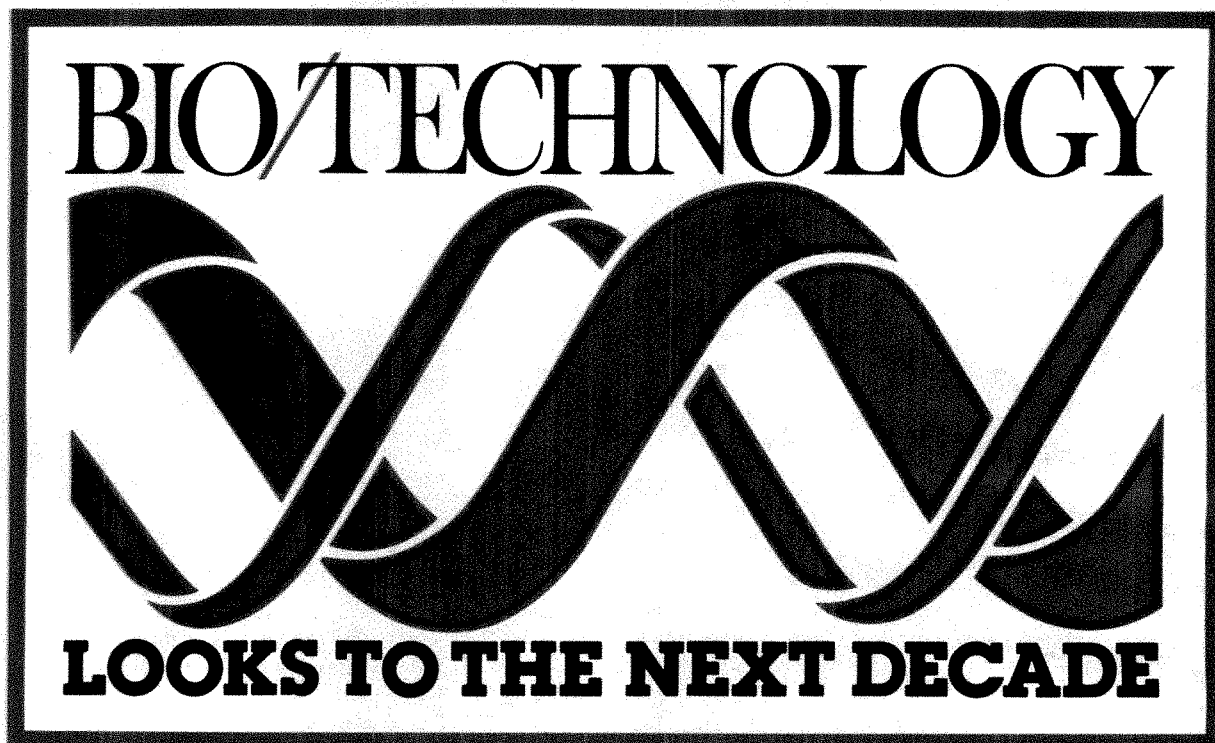
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THE MOLECULAR BIOLOGY OF PLANTS AND ANIMALS: A VIEW FROM THE FRONTIER

SPEAKERS:

David Goeddel

Director, Molecular Biology, Genentech

Richard Flavell

President, Biogen Research Corp.

Norman Borlaug

CIMMYT, Mexico City and Professor, Dept. of Soil and Crop Science, Texas A&M

Robert Fraley

Manager of Plant Molecular Biology, Monsanto

David Evans

Vice President for Research, DNA Plant Technology, Inc.

Charles Arntzen

Associate Director of Central Research and Development, E.I. Du Pont de Nemours and Co.

JANUARY 20—AFTERNOON

SEMINAR IN MOLECULAR BIOLOGY

PANELISTS

David Goeddel, Co-Chairman

Richard Flavell, Co-Chairman

Budd Colby

Director, Technology Development, Triton Biosciences

SEMINAR IN AGRICULTURAL BIOTECHNOLOGY

PANELISTS

Norman Borlaug, Chairman

JANUARY 21—MORNING

COMMERCIAL PRODUCTION PROCESSES

SPEAKERS:

Malcolm Lilly

Professor of Biochemical Engineering, University College London

Robert Herschberg

Department of Process Development, Genentech

Yukio Sugino, Director

Biotechnology Labs, Takeda Chemical, Japan

Ephraim Katchalski-Katzir

Professor of Biophysics and Biotechnology, Tel Aviv University, Israel

Where has all the stuffing gone?

British universities seem ill-equipped to fight back against their most obdurate critic, the government whose instrument of policy on higher education they are supposed to be.

BRITISH universities have had a rotten time in the past few years. Is it possible that their painful experiences have made them incapable of resisting the continuing pressure from their critics, chiefly the British government? That is the charitable explanation of the mood of last week's conference, organized by the universities' trade association, the Committee of Vice-Chancellors and Principals, which was ostensibly intended as a forum for hammering out a reply to the government's green paper on higher education, published earlier this year (see *Nature* 315, 265; 1985). That the committee should have it in mind to reply to the government's tactless document by producing the one that should have been published, cheeky though it may be, is sound. The danger is that the result will not be a defence but a kind of apology, like that of the legendary servant girl who was in trouble for having produced a child of unknown parentage and whose defence was along the lines of "but it's only a small one".

To be fair, the universities seem to have taken account of one of the features of the British educational system that cries out for reform. Several speakers at last week's conference spoke of the need for a broader curriculum both at the secondary schools from which the universities recruit their students and even within universities themselves. It is high time, in the apparently endless brooding about the failure of the British system of higher education, that more serious attention was paid to the value of a liberal education. Indeed, the need that something should be done to make British education less narrow has been urgent at least since the rapid expansion of the early 1960s. For much of that time, British academics have been chief among those seeking to resist change. Only reluctantly, in the past few years, have they been won round to a reform of the pattern of school education that will allow a modest broadening of the education of intending students, no doubt in part because they appreciate that it cannot harm the university system if it becomes more common that students should follow four-year undergraduate courses (as they do already in Scotland). The trend is nevertheless a sign of grace.

Research

On research, the universities seem less sure of themselves. The tenor of the British government's complaint is that too much of what passes for research at British universities is academic in character, unlikely to contribute to national prosperity. This is the spirit in which the budget cuts have been put forward as beneficial incentives to be more practical. Driving academics looking for funds into the arms of industry is bound to make their work more directly relevant to national prosperity, the argument goes. The reply on behalf of the universities last week was curiously muted. Universities have a snobbish disdain for industry (as the green paper complains)? "That cannot be true now; just look at the difficulty we have in persuading graduates to stay on for research!" Universities have been slow to assist with the transfer of technology to industry (another complaint)? "Ah well, things are changing; some universities are making substantial sums of money out of industrial liaisons." The weakness in such replies is that they concede the principles of the complaints the government has been making. It would be permissible, and in the end rather safer, to insist that only individual

universities can usefully shape their own destiny.

What more robust defence can there be? What British universities should recognize is that there is no reason why the system of higher education to which they collectively belong should be capable both of contributing to the development of technology and of preserving the old traditions of scholarship and research, but that the flaw in the British government's complaints over the past six years is that it is unreasonable to expect that each single institution will contribute in the same way. Constitutionally, the vice-chancellors' committee cannot easily abandon the polite convention that all universities are equal, which is especially crippling when it is acknowledged that the essence of an effective reply to the British government's complaints must be a plea that greater diversity is now, as for decades, the most urgent need. The truth is that British higher education has been made too uniform as a consequence of government policies and the polite conventions of the academic life. Only within such a framework will it be feasible for some universities and polytechnics to make their way in the world by concentrating on the needs of industry and for others to do what they already do well, scholarship and academic research. But will not greater diversity flow from the plan of the University Grants Committee to begin allocating a part of its subvention for universities on the basis of a judgement of the quality of the research which is at present carried out? The first steps in this direction will have been taken six months or so from now. The most obvious danger is that the result will be to strengthen the universities that are already strong, and publicly marking out the others as second-rate, undeserving of more than basic support and thus incapable of self-improvement.

Budget

The impending row about this discriminatory allocation of money is only one of the several novel crises now facing British universities. Another is the continuing decline of the budget, estimated to be shrinking still at between one and a half and two per cent a year when allowance is made for increased costs and the difficulty of settling salary increases within the government's national allowance. The University Grants Committee has said that it will not be long before it is forced to recommend that one or more institutions should be closed, but that it will not shoulder the invidious task of saying where the axe should fall. That crisis could come sooner rather than later, perhaps as early as next May. Then, to round out the sense of gathering gloom, the function of the grants committee itself is being reviewed. The excuse is a minor reference in a report on the efficiency of universities published earlier this year, but it seems unlikely that the committee appointed for the purpose will constrain itself within a framework of accountancy. The ideal is that the review should settle on principles for supporting all kinds of higher education, polytechnics as well as universities, and should then work out a way in which the grants committee can function more as a monitor of success or failure than as a source of funds. But who will say what is success? On last week's showing, the vice-chancellors are not up to the job. Nor, by the evidence of this year's green paper, is the British government. Might that not be the lasting function of a grants committee with a broader membership? □

Crossing the water

France and Britain now seem bent on building a physical link. Will it really happen?

THE bids announced last week by the consortia wishing to build a physical link between Britain and France have been widely predicted and are, as far as they go, staid and safe. The Channel Tunnel Company, which already owns a number of holes in the ground and beneath the seabed on the English side of the water, is understandably bent on building a tunnel, a rather better one than that abandoned in the early 1970s. Others are variously concerned to build a bridge the whole way across this turbulent 21 miles of water, or to span just over half of it with bridge-like structures that then disappear beneath the sea in a stretch of tunnel that will allow shipping to pass without hindrance. The tunnel, which would accommodate only trains, is the cheapest project (at roughly £2,500 million). The bridge-tunnel plan is roughly twice as expensive; building a bridge the whole way across (with an accompanying railway tunnel with which to placate the nationalized railway systems on each side of the Channel) works out at half as much again. The only certainty about what will happen next is that the governments of France and Britain will decide on the scheme to be allowed to go ahead, but that the construction costs will have to be met by private interests, banks and the like.

So has the time arrived when the future of the Channel tunnel link will be laid to rest? Most probably not. For the best part of two centuries, schemes to build a Channel link have been canvassed enthusiastically and, then, as suddenly abandoned. The fear that Napoleon might be able to march an army through a tunnel brought the first wave of enthusiasm to a halt. Now, not even fears of cultural invasion can upset the case for building a link of some kind across the Channel, which is crudely economic. All the bidders now in the market for the attention of their governments base their arguments on the assumption that the cross-Channel business will be worth £500 million a year or so by the time that a tunnel or a bridge is built. If the estimates of cost now put forward are anything like correct, each of the schemes should be economically viable at some point in the 1990s. But how likely are the planners to be proved correct? And what will happen if their estimates are seriously in error, and if the project chosen in the next few months runs into trouble, geological or financial, in the succeeding years?

Whatever the two governments may say, they cannot wash their hands of some kind of responsibility for whichever project they choose. From the start, they will be intimately involved, as governments, in the renegotiation of navigation rules between France and Britain. And on each side of the Channel, the project will need planning permission on a scale that only governments can provide. Then, in reality, there is no chance that either government could turn its back if a project turned sour after a few years. At the very least, it might be necessary to dismantle a few surplus structures in the centre of a busy international waterway. But the political pressures that would apply to government that decided it could abandon a physical link half-way through construction would be irresistible.

That is one reason why there is the strongest case for the two governments to face the responsibilities they will not be able to escape now, and not to wait until the project is under way, with money committed. It is right and proper that they should expect the bulk of the funds required for the project to be raised from private sources, but it would help to give the project solid foundations if the two governments were to become minority shareholders in their own right. Then, if the worst came to the worst, they would be in a position to decide whether to step in to mount a rescue for a faltering project or to continue to stand aside.

Meanwhile, it remains to be seen which project will be chosen. Given their mutual interests in the prosperity of railways, both governments have a vested interest in the rail tunnel, but this should be suppressed. The high economic value of the

traffic between Britain and France, and the pace at which it is growing, shows that the trade transcends the narrow interests of the railway industries. To be able to drive across as well as go by train should be the objective, whence the case for the hybrid bridge and tunnel at the very least. The misfortune in all this, however, is that so little has been done to implement what is obviously the best of all ways of crossing the Channel, the scheme for putting the clock back to the late Pleistocene, when lots of people crossed from France to Britain, and building a dam across this narrow strip of water, nowhere more than 120 feet deep. □

No tin-pan alley

The International Tin Council, an international cartel, is in trouble. The surprise is the delay.

Two weeks ago, the London Metal Exchange was thrown into chaos by the announcement that the gentleman called the buffer stock manager of the International Tin Council had run out of money. Trading in tin was promptly suspended, and has not yet been resumed. Meanwhile, commodity brokers in the City of London, used as they are to skating on thin ice, are worried sick by the prospect that some of them may not be able to pay the debts they have incurred over the past several months in buying and selling tin. Even the British government, in its avuncular role as the custodian of the last resort of the reputation of the City for honest dealing, is alarmed that the reputations of people and of institutions may be so damaged that people elsewhere will not use London as a place in which to buy and sell metals and other commodities. (London has a virtual monopoly on metal-trading.) The several causes of the tin crisis have by now been well catalogued. Improbably, they range from the illicit activities of poachers based in Singapore who have been dredging tin from the deep waters of Malaysia to the cupidity of the tin-producing countries, which have done everything they can to keep up the retail price of tin, including the curious practice of buying back through the Tin Council roughly a third of all the metal their producers have been able to sell. Collectively, the tin producers and their customers have been united through the International Tin Agreements, by whose terms the International Tin Council exists, in an attempt to ensure that water runs uphill. In the past few days, the natural laws have reasserted themselves.

Nobody should be a whit surprised. All along, the objective of the Tin Council has been to stabilize the price of tin. The buffer stock manager has instructions to buy the metal when the price falls below a certain value (fixed periodically) and to sell when the price exceeds another value. In between, however, he is neutral, sitting on the stocks he has accumulated. Although the United States has remained stolidly outside the tin agreements, other consumers have over the years benefited almost as much as the tin producers. Prices have indeed been stable, much more than might be expected in a market in which the balance between supply and demand can fluctuate enormously. The trouble is that, as in the petroleum market, stability means different things to producers and consumers. In tin, the chief producers, among whom Malaysia is the most important, have naturally taken the view that stability is not worthwhile unless it also implies a high price, just over £8,000 a tonne when trading was halted ten days ago. But the industrial demand for tin has shrunk as the many importers of this expensive metal have learned to use others instead. In retrospect, it is clear that the buffer stock manager should have blown the whistle earlier, and that he should not have waited until he ran out of credit with the banks to whom the tin market now owes the best part of £600 million. Rarely can the buffer stock have grown as consistently as in the past few months. But the diagnosis is easier after the event. And now, when nothing is sure except that a great many people and banks will be left holding a large quantity of tin they do not want, the lesson to be learned from this discreditable episode is that the laws of supply and demand are not safely defied. □

AIDS

Politics of premature French claim of cure

THE French physician who claimed last week a dramatic improvement in patients with acquired immune deficiency syndrome (AIDS) when they were treated with the immunosuppressant cyclosporin A now says he was pressed into a premature press conference by an over-enthusiastic minister.

"It wasn't our decision — it was the ministry of health's", said Dr Philippe Even of the Laennec Hospital in Paris. Dr Even agrees that the announcement of results after a week's trial with six patients, without controls, was "premature", but claims it was the pressure and the enthusiasm of health minister Mme Georgina Dufoix that led to the press conference. Dufoix appeared on television to claim this was "a great French discovery" that "bears the label of France".

Even is now following the line of his critics, saying that what is needed now is a full-scale trial. Ironically, it was his request to the ministry for help in setting up a trial that led to the widely-condemned public announcement.

Nevertheless, Even says, the treatment of his key patient, a 38-year-old man acutely ill with "end-stage" AIDS, has been "really dramatic". This person had been the most seriously ill of six patients treated, by last Friday, for nine days with the drug. The patient has shown a 100-fold increase in the population of T-4 (T-helper) cells, the white blood cells specifically infected by the AIDS virus. Now the man has a T-4 cell count of 400 per cubic millimetre, against 4 previously. Having been comatose and bed-ridden, he is now walking and feeding himself.

Dr Even also claims that "something very curious" happened during treatment indicating that cyclosporin A has a genuine effect. The patient, who had been taking cyclosporin orally got diarrhoea on the sixth day of treatment which interfered with absorption. His T-cell count immediately dropped, so that Even and his colleagues Jean-Marie Andrieu and Alain Venet decided to administer the drug intravenously. "Immediately his T-cell count rose again", says Even.

The five other patients, all less acutely affected, had shown at least a doubling in T-cell count since treatment to levels of more than 300 per cubic millimetre (against a healthy level of some 700–1,200).

Even's explanation is that cyclosporin A halts the activation of T-4 cells but does not inhibit the production of new T-4 cells by the bone marrow and the thymus gland. The result, says Even, is that mature T-4 cells can be held in check while

the immune system regenerates itself. Even says that the activation of T-4 cells is "pivotal" in the replication of AIDS virus and the development of the disease; he speculates that an occasional course of cyclosporin A might keep the disease in check or even, with luck, eliminate it.

Outside France, AIDS clinicians say that the premature release of results was not only improper but cruel: "some of our patients will clutch at anything". There is also a fear that cyclosporin A could in the long run actually exacerbate the disease. According to one leading immunologist, the drug alone mimics the immunosuppressive effects of AIDS itself. There are also cases of AIDS-antibody-positive renal transplant patients who were put on immunosuppressants subsequently developing more acute AIDS than patients who had not been immunosuppressed, it is pointed out in London.

Moreover, the rise in T-4 cell count seen in Paris appears too rapid to be explained by regeneration of lymphocytes by the

bone marrow, a process that might be expected to take 3–4 weeks, rather than just nine days. Further, the cerebral infections seen in AIDS patients, perhaps mimicking the infections of sheep by visna virus, may be resistant to cyclosporin A. London immunologists point out. The target cells for the HTLV-III/LAV virus of AIDS in the brain have not yet been identified, and may not be controlled by cyclosporin A even if it crosses the blood/brain barrier.

But Dr Even says that not one in five of the population of new T-cells in the patient's blood is an immature T-6 cell from the thymus, indicating that cyclosporin A has stimulated the production of new lymphocytes, at least from the thymus gland.

The next step, says Even, will be the setting up of a multi-centre trial of cyclosporin A in acute patients in France and, later, of an international trial. The pharmaceuticals company Sandoz, which makes and markets cyclosporin A, is convening an international conference on 8 November at its Basle headquarters in Switzerland, to prepare just such an international trial. But the only North American delegates attending will be Canadian, Even says. So far there are no delegates from the United States, and therefore no apparent US interest in joining in a trial. "That is their decision", Even says.

Robert Walgate

SDI

Star wars critics criticized

Washington

THE US Congress's Office of Technology Assessment (OTA), no stranger to controversy over President Reagan's Strategic Defense Initiative (SDI), has run into yet another dispute on the subject. Dr Robert Jastrow, an independent authority on missile defence, and Dr Frederick Seitz, chairman of the Defense Science Board, last week protested that the majority of an OTA advisory panel that supervised a recent OTA report* on SDI (see *Nature* 26 September, p. 276) were "strongly opposed" to SDI, and that OTA staff were biased against the project.

One of Jastrow and Seitz's chief complaints is that the OTA advisory panel, of which Seitz was a member, did not vote to endorse the report, and that there was no outside review. OTA's report concluded that SDI will increase national security only if the project achieves great technical success and if the Soviet Union cooperates in a negotiated arms reduction. Peter Sharfman, OTA's international security programme manager, replies to the criticism by pointing out that OTA's advisory panels never vote on staff reports and that their function is to provide independent review.

The Jastrow/Seitz attack on OTA was made at a briefing held by the Heritage Foundation, a conservative think-tank with strong links with the Reagan adminis-

tration. Seitz was recruited to the advisory panel after General Daniel Graham, a strong advocate of SDI, had resigned, also alleging bias. Sharfman rejects categorically the charge that a majority of the panel were "strongly opposed" to SDI, and denies the suggestion that OTA staff were stretched beyond their limits.

Apart from the procedural issues, Seitz and Jastrow say that OTA failed to take account of numerous technical advances in missile defence in the past year. In their view, progress has been so encouraging that key demonstrations of SDI technology might be feasible 10 years sooner than was expected, with deployment possible during the 1990s. Among the advances named are the free electron laser, electromagnetic railguns and a new type of rocket engine, SCRAMJET.

OTA replies that it was familiar with the technological advances but avoided giving performance details because they are classified; all the technologies mentioned by Seitz and Jastrow, excluding SCRAMJET, are described in the OTA report. And in reply to a criticism that OTA overestimated the effectiveness of Soviet countermeasures, OTA says it regrets that specific technical objections were not raised by the advisory panel before the report's publication.

Tim Beardsley

*Ballistic Missile Defense Technologies. Office of Technology Assessment, 1985.

Export controls

Scepticism over liberality promise

Washington

THE US Department of Commerce (DoC), long the bugbear of US high technology exporters because of its allegedly inefficient processing of export licence applications, is to make substantial changes in its licensing regulations. The changes are required by recent legislation and by agreements reached with the United States' partners in COCOM, the Coordinating Committee on Multilateral export Controls*.

Most recently, the department has been advertising to industry the virtues of its new "foreign availability office", intended to ensure that the United States does not restrict on national security grounds exports of high technology items that are freely available to the Eastern bloc from other sources. But, logical though this sounds, industry is sceptical of whether the office will have the desired effect.

Foreign availability was supposed to be taken into account in determining whether to allow exports even before the Export Administration Amendments Act became law on 12 July, but requests for licences on these grounds usually fell on deaf ears. The new office is intended to change that. William Archey, assistant secretary for trade administration, has described the new foreign availability rules as "the dark horse" of export regulations.

Under the new act, after receiving an export licence application that rests on a claim of foreign availability, DoC will be allowed a grace period of 18 months during which time the US government can seek to "negotiate away" the foreign source of the item in question. The exporter has to establish that the item is available in comparable quantity and quality from a foreign source, but the burden of proof will now lie with DoC if it seeks to reject the applications. Even then, DoC may not be able politically to allow the export of some items demonstrably of foreign availability. Yet, even if the cynics are right, the office may still benefit industry in other ways.

Bill Maxwell, of the Computer and Business Equipment Manufacturers' Association, believes that the new office will probably succeed in preventing items being added unnecessarily to DoC's "commodity control list" of proscribed items, even if it does not manage to remove any already there. And that could be significant, especially with increasing sophistication of electronic equipment manufactured in some other "Pacific rim" countries.

The United States has consistently made the running in efforts to restrict the flow of Western high technology equipment to the East. Last year, DoC turned down licence applications for \$300 million worth of exports for which orders had been received, on the basis of pre-licence

checks and intelligence reports. But the United States unilaterally controls exports of some items that are not controlled by its major trading partners, which has frequently led to quarrels with allies.

One especially sensitive issue has been whether contracts legally entered into can subsequently be terminated for foreign policy reasons by invoking export controls. Despite lobbying by allied governments, nothing in the new act alters the presumption of extraterritoriality. The new act will nevertheless make it slightly harder for the President to terminate contracts for foreign policy reasons. He has to testify that the controls are necessary, and that a failure to act will lead to a "breach of the peace". (What exactly constitutes a breach of the peace is left undefined. The resulting loophole is "big enough to drive a truck through", according to one independent specialist.)

Among COCOM's more significant achievements was the agreement last year to decontrol exports to the Eastern bloc of many less-capable computers, on the grounds either that they were unlikely to be of military use or that control was impractical. COCOM merely has to be notified when exports of this sort are planned, and DoC has recently introduced regulations to allow such exports from the United States to COCOM member countries without a licence.

But if the decontrolled items will reduce DoC's workload, other changes required by the new act will increase it. DoC has been given tight new timetables for processing licence applications: 15 days for exports to COCOM countries and 60 for most others for which COCOM review is necessary (down from 90). The timetables will benefit US exporters — if they are met.

Walter Olson, deputy assistant secretary for trade administration, says the United States is "pleased" by progress in COCOM, although he notes there are still areas where the United States and other members do not yet see eye to eye.

Disputed items include certain kinds of software, particularly that used in designing and fabricating semiconductors. But Olson hopes that further progress will be forthcoming, especially now that COCOM has changed its cycle of operations so that one quarter of the entire (secret) proscribed list will be reviewed each year, rather than there being a complete review every three or four years. The result, he says, will be that DoC will be able to keep effective controls on those items that need to be safeguarded, while ensuring that some of the complaints that have been heard in the past about US manufacturers hobbled by harsh export controls are not repeated.

Tim Beardsley

* COCOM member countries are the United States, the United Kingdom, France, Belgium, Italy, the Netherlands, Luxembourg, Norway, Denmark, Canada, West Germany, Portugal, Greece, Turkey and Japan.

Texas keeps on bidding for collider

Washington

COMPETITION among states to play host to the proposed Superconducting Supercollider (SSC) has led to questions in Congress about design assumptions even before a complete conceptual design has been drawn up. When the central design group announced in September its choice of magnet design for the accelerator, most people thought the question had been settled. The group chose a high-field cos θ design over a competing superferric design (see *Nature* 26 September, p.277). But Representative Joe Barton (Republican, Texas) has now carried out his own study, and concludes that the design team made a mistake.

Both designs employ superconducting magnets, but where the high-field cos θ design relies on the arrangement of the current-carrying wires to produce a uniform magnetic field, the superferric design relies partly on steel pole pieces to shape the field and return flux efficiently. The superferric magnet design is cheaper per foot, but because it produces a lower-strength field more magnets are needed. One important factor in a choice between the two designs is the cost of tunnelling, which depends on the terrain.

The superferric design would lead to a

larger SSC with a circumference of 100 miles, as opposed to a modest 60 miles with high-field magnets. Texas would be ideal for SSC, especially if the larger version were to be built, for the state has sites where tunnelling costs would be low. Last week, at a hearing in the House of Representatives on prospects for high-energy physics, Mr Barton told Maury Tigner, head of the design group, that "it could be shown that" the superferric design would lead to a cheaper SSC. Tigner hotly disagreed, offering to debate with Barton, point by point, on the magnet selection panel's analysis. Barton said he might submit some written questions.

With Congress in a deficit-cutting mood, winning a commitment to build SSC will be difficult. The US high-energy physics community last week brought in big guns from Europe to help make their case, in the form of Herwig Schopper, director of CERN in Switzerland, and Carlo Rubbia, at present at Harvard University. Present estimates of the cost of SSC, a 40 TeV proton-proton collider, range from \$3,000 to \$6,000 million. The design group should complete its conceptual design study in April, when more accurate cost estimates should be possible.

Tim Beardsley

Plant engineering

Gene transfer makes a start

Savannah, Georgia

ONE reflection of the rapid growth of plant molecular biology is that it is now possible to attract some 1,800 participants to one of those unwieldy international symposia whose concurrent sessions occupy the ballrooms of the larger hotels in out-of-season resorts. The International Society for Plant Molecular Biology at any rate regards its congress held here last week as a milestone in the field.

Although the use of recombinant DNA to transfer genes between plants may eventually change the face of the Earth, to judge from last week's scientific presentations, its principal impact in the immediate future is likely to be on the fortunes of the agrichemical industries that support much of the basic research. Only two weeks ago, a team from Calgene reported (*Nature* 317, 741; 1985) the introduction into a tobacco plant of a bacterial gene conferring resistance to the Monsanto herbicide glyphosate; similar results were reported here by Rob Fraley (from Monsanto's own laboratories working in collaboration with Nam-Hai Chua and colleagues at Rockefeller University), but with a plant gene and in tomato as well as tobacco and petunia.

Glyphosate is an indiscriminate herbicide that destroys crop plants and weeds alike, and whose use is therefore confined to ground clearance. Glyphosate tolerance into crop plants would thus greatly extend the application of the chemical.

But this is not the sole, or probably even the principal, reason for the impressive advances in the manipulation of herbicide resistance. Analogous experiments were reported by Lawrence Bogorod (Harvard) with a gene for the Ciba-Geigy herbicide atrazine, which is now out of patent and so no longer of compelling commercial interest. The attraction is that the genetic and biochemical basis of herbicide tolerance is both relatively simple and extremely well understood. Most broadly desirable genetic assets, such as the ability to resist pathogens or fix nitrogen are complex and relatively ill-understood. In pathogen resistance, pathogens seem capable of producing virulent mutants about as fast as molecular biologists can engineer plants to resist them. Herbicides are an easier target.

Among other sobering considerations offered in a presentation by M.S. Swaminathan, director of the Institute of Rice Research in Manila, is that the basic genetics of Third-World staples such as rice are in such a primitive state that they have simply not been accessible to molecular manipulation. A further difficulty is that rice, like other cereals, is a monocotyledon. So far, the recipes for regenerating complete plants from manipulated cells will work only for dicots, whence the con-

centration on tobacco, petunias or, at best, tomatoes.

Nevertheless, the Rockefeller Foundation is supporting a campaign on the rice genome which, with the use of the molecular mapping techniques that have been so successfully exploited in the analysis of human genetic disease, should be reasonably well charted within a year or so. Progress on regenerating genetically engineered plants is less predictable (tissue culture is still largely a black art) but two speakers at Savannah reported a significant step forward. Genes artificially introduced have not in the past functioned stably in cultured cells from monocotyledons, but this has now been achieved in maize (Michael Fromm, Stanford) and Italian rye grass (Lawrence Potrykus, Friedrich Miescher Institute). The next step is to persuade the cultured cells to

regenerate into a complete plant, which many believe will be a matter chiefly of luck and patience.

Meanwhile, new techniques for gene transfer based on electroporation (which allows DNA to enter cells exposed to strong electric fields) are helping molecular biologists to identify and isolate the genetic control elements that determine the responses of plants to the critical features of their environment. The DNA elements that enable genes to be activated by heat and light have already been identified; Jim Peacock reported that his group at the Commonwealth Scientific and Industrial Research Organization in Canberra now has a gene that controls the induction of others in anaerobic conditions. In principle, these genetic control elements could be manipulated to increase the resistance of crops to natural disasters such as drought. How it will be in practice, and when, it is too soon to guess. Even resistance to herbicides is not yet ready for the field. **Miranda Robertson**

Space insurance

Market confident of survival

Washington

US GOVERNMENT intervention to provide emergency insurance cover for commercial space ventures would be disastrous. Witnesses from the National Aeronautics and Space Administration (NASA), insurance companies and satellite manufacturers all agreed on this point when telling Congress last week how to prevent a space insurance crisis; more than \$600 million has been lost by insurers in the past 21 months, threatening the much vaunted commercialization of space.

James Barrett of Intec (International Technology Underwriters) is confident that market forces will prevail and allow the insurance industry to continue to "facilitate, but not subsidize" commercial space projects. He suggests spreading the load of space insurance risks throughout many smaller companies, which could support an "insurance pool" dedicated to space shuttle launches and cargoes. He also feels that the manufacturers should take more of the risk for the functioning of their products, which would doubtless improve reliability. Barrett is opposed to the sort of competition that is occurring at the moment between Arianespace and NASA; because Arianespace provides guaranteed launch insurance at below-market rates, NASA is planning to offer cut-price relaunches to customers whose satellites have failed within a given timespan. Such deals will deprive the insurance industry of premium revenue and inhibit the spread of risk, according to Barrett.

Jack O'Brien, NASA's general counsel, agrees that the market will stabilize without government intervention. NASA

already helps its smaller customers by waiving liability for small self-contained payloads so that the only loss the experimenters face is the face value of the project. NASA also disallows claims against each other by participants in a particular flight, and includes itself in this ban. It is carrying out a further evaluation of its risk allocation policies, but wants to find a solution in conjunction with the insurance industry that does not open the door for government regulations.

Predictably, the manufacturers themselves do not want to be liable for any loss risk. Charles Schmidt of RCA Astro-Electronics says that the fiercely competitive business leaves no room for such guarantees. Smaller companies would lose business if a "no insurance, no financing" policy were introduced. One compromise would be for the industry itself to form a last-resort pool.

The message to the government from the construction industry and the insurers alike is clear — the market will look after itself. The satellite industry was so successful in the first ten years of its existence that insurers have grossly underestimated the cost of risks, leading directly to the present crisis. Underwriters are convinced that the pendulum will swing back and that a stable compromise can be reached if the government will give them time. In a country where space is taken so seriously that the normally conscientious chairman, Bill Nielson (Democrat, Florida), of the committee taking last week's evidence was absent because he is in training for the next shuttle flight, the commercial space industry had better make sure it has reason for its confidence that a solution can be found. **Maxine Clarke**

Japanese conservation

War on war on migrating birds

Tokyo

JAPAN may not have much of reputation abroad when it comes to protection of wild life. But inside the country, conservation groups are growing stronger. In the coming weeks, volunteers from the Wild Bird Society of Japan will be taking to the hills in force to try to prevent the mass netting of migratory birds.

During middle to late November, migrating birds pour into northern Japan to escape the Siberian winter. In olden days, many were netted out of sheer necessity;



the farmers of the northern provinces were poor and the birds provided one of the few sources of protein in winter. Now that Japan is an advanced technological power, poverty has disappeared and there are not many people left in the bird netting business. But advanced technology has also made it possible for a few people to catch an enormous number of birds.

Nowadays, Japan makes the world's finest mist nets — light, strong and so fine that they are invisible to the bird's eye — and plenty of inexpensive audio systems that can be used to broadcast recorded bird calls. The result is that flocks of thousands of birds may be drawn into a large net and captured at one time. Those most frequently taken are *tsugumi* (dusky thrush, *Turdus naumanni*), *kashiradaka* (rustic bunting, *Emberiza rustica*) and *atori* (brambling, *Fringilla montifringilla*). Last year alone it is estimated that more than four million birds were killed and mostly sold to restaurants. And as a thrush can fetch as much as 1,000 yen (nearly US \$5) it is a profitable business. Recently it has attracted the interest of criminal gangs.

The setting of mist nets has actually been illegal since 1947, but as there is no restriction on their sale and fines for illegal taking of birds are small, the law has had little effect.

Increased concern for wild birds is now beginning to make inroads into the poachers' takings. The membership of the Wild Bird Society of Japan has increased

seven or eight times since the end of the 1970s to its present 16,000. This year, for the first time, volunteers from the society will patrol the hills all through the key November weeks, searching for illegal mist nets. If any are found, the evidence is guarded and the police called.

The society would like to take things a step further and ban manufacture of mist nets altogether. That would please environmental groups in Italy and Spain which are fighting a mass capture of migrating birds in their own countries. Mist nets are imported from Japan. The Environment Agency, which has been pressing local governments to crack down on the poachers, is also backing a ban on mist nets. But the agency lacks regulatory powers and has yet to move the ministry responsible, the Ministry for International Trade and Industry (MITI). So far, MITI has tended to take the manufacturers' side, for a variety of rather spurious reasons, including the possible effect of a mist net ban on the hair net industry.

Alun Anderson

New Soviet guidelines

THE new draft programme of the Communist Party of the Soviet Union, the first since 1961, includes not only a strong commitment to scientific and technical progress, but also a code of conduct for scientists.

The party, it is stated, "supports the bold quest, the rivalry of ideas and approaches in science, and fruitful debates and discussions". Contraindications for science therefore include: scholastic deliberations, the passive recording of facts without drawing bold generalizations, jumping on bandwagons, and severing oneself from reality. In view of the complex and interconnected nature of current problems, a deepening of the links between the natural, technical and social sciences is required. Forms of organization of science should be developed that will facilitate interdisciplinary study of topical problems and the necessary mobility of scientific cadres. The structure of scientific institutions and of research and development procedures must be made more flexible.

An "essential condition for the progress of science", it is noted, is the constant influx of fresh forces, including forces from the production sector, and the skilful utilization of scientists' creative potential.

Finally, and tacitly admitting that ideology is not enough to get and keep Soviet research working at full capacity, the programme specifically urges the provision of financial incentives for scientists "depending on their real contribution to the study of theoretical and applied problems".

Vera Rich

French ceramics

Plans for silicon carbide fibres

HERMES, France's answer to the US space shuttle, will be made of ceramic composite, and will thus avoid the sticky tiles that caused complications in the early days of the US craft. At least, those are the plans of Société Européenne de Propulsion (SEP), the company charged with preparing the curved nose, leading edge of the wings and underbody of Hermes, the parts that will face re-entry temperatures of up to 1,600 degrees centigrade for periods of some 20 minutes.

SEP has chosen for the task one of its most advanced ceramics, consisting of silicon carbide fibres bonded into a sintered silicon carbide matrix: "a kind of ceramic concrete", according to one British ceramicist. A major problem with such materials is to produce curved forms, and Hermes is of course full of aerodynamic curves. But SEP has been working on similar composites, including carbon-carbon matrices, for a decade, and has already produced trial curved silicon carbide/silicon carbide sheets some 50cm-1m across. This is a long way from the many metres required for Hermes sheets, but SEP reckons it has another ten years to develop the technology. If all goes well with the development, the ceramic will be more than mere cladding and will act as a structural part of the craft, although it may need support from more conventional stress-bearing structures underneath.

Meanwhile, according to M. Roger Lesgards, president of SEP, the company's first priority will be to establish French independence in the supply of the silicon carbide fibres, which at present come from Japan. SEP intends investing jointly with the French chemicals giant Rhône Poulenc in a factory to produce the fibres and the composite. SEP did the same with carbon fibres a year ago, in setting up a factory near Lyons to manufacture the fibres in concert with the steel company Alsthom Atlantique.

Lesgards describes SEP as having a "really good position" in the world market for high thermal resistance composite ceramics. Japan asked SEP for samples of the silicon carbide/silicon carbide matrix, says Lesgards, and the US Department of Defense is interested in using the material for missile nose-cones. The composite is already in use as brake disks for Formula One racing cars, and is also being tested for use as vanes to direct the thrust of rocket motors.

SEP foresees three possible world markets for the material. The first, requiring production levels of a few tonnes per year, would be a market for space and military uses. The second, raising the market tenfold, would be in aeronautics (for example, in turbines). The third and largest,

involving another tenfold increase, would involve uses in diesel motors and other common equipment, which could raise demand to a few hundred tonnes a year. Lesgards estimates.

The material is costly to produce, but economies of scale could reduce cost tenfold at the highest market levels. Lesgards predicts. With this in mind, SEP recently entered into cooperation with the German diesel engine manufacturers MAN to develop ceramics for diesel motors, with support from French and German government agencies.

SEP also plans to extend its international cooperation to work on ceramic turbines with companies in Norway, Sweden, Spain and Italy, if support is forthcoming from this week's ministerial meeting on Eureka at Hannover. Under Eureka, SEP has put forward a plan for a FF 200 million (£18 million) research programme on ceramic turbines. The company has also proposed the development of a commercial demonstration ceramic diesel engine (FF 100 million) and two varieties of ceramic turbine (FF 100 million each). SEP would be happy if half of the total FF 500 million were supported by governments or banks, Lesgards says. **Robert Walgate**

British nuclear research

Harwell goes half-private

In the UK Atomic Energy Authority (AEA), 1 April 1986 is known as Vesting Day. This is Civil-Service-speak for the day on which AEA ceases to be a division of a government department and becomes a "trading fund", equivalent in status to that of a limited company in which the British Treasury is the only shareholder. Among other things, the authority will thus be able to lend and borrow money on the open market. The Treasury will set financial targets, and there will be high hopes on the government side that the authority will be able to generate a surplus.

AEA's largest establishment, the research laboratory at Harwell in Oxfordshire, is also the best placed to profit from the new regime. A year ago, an encouraging 33 per cent of its research was supported by non-governmental contracts, but now the figure is nearly 60 per cent.

To continue at its current level of activity, Harwell needs funds totalling £100 million a year. From next April, there will no longer be a subvention from the De-

partment of Energy (DoE) to cover research carried out on behalf of government departments and other parts of AEA. Instead, separate contracts will be negotiated to cover each research programme — including fast reactor research, fusion research and work on thermal reactor and general safety research. It does not follow that the individual contracts will together compensate for the lost lump-sum contribution, the two being calculated in different ways, which is another stimulus for Harwell to look further afield for contracts.

Recent investment in the facilities at Harwell should help in the push for more outside contracts. A new radiochemical handling laboratory has been built, based on experience gained at Los Alamos in the United States and at Karlsruhe and Jülich in West Germany. The five high-integrity concrete cells can contain any combination of alpha, beta and gamma radiation. Each cell is ventilated separately so that a chemist measuring, say, a caesium isotope in one cell need not fear that someone else's experiment is generating just that activity at the other end of the containment area. Experiments are set up in large stainless steel boxes, or "cassettes", before being hoisted into the containment cell on cranes.

The new laboratory was opened last week by Sir Frederick Dainton, on his last day as chairman of the National Radiological Protection Board. The date was chosen to mark an anniversary of sorts — 40 years ago, on 29 October 1945, Prime Minister Clement Attlee announced in the House of Commons that Harwell, a disused airfield near Didcot, was to become Britain's first nuclear research laboratory.

Main customers for the new facilities will be British Nuclear Fuels Limited, the Central Electricity Generating Board, other AEA divisions and Amersham International, the radiochemicals company spun off from AEA several years ago. Although the company is now branching out into the increasingly popular field of non-radioactive assay kits, Amersham's isotope business is still large, and will be an important part of Harwell's plans, managed through Amersham's own offices on the Harwell site.

While Harwell can contemplate a future of at least the same level of activity as now — provided that in the long term it is able to adapt to the changing needs of industry — some other AEA divisions have fared less well in recent government reviews. Spending on fusion research at the Culham laboratory, for instance, is to be reduced by £1 million, £2 million and £3 million in three successive financial years. However, support for the Joint European Torus (JET), also sited at Culham, is not affected. **Charles Wenz**

New Soviet space agency is formed

THE Soviet Union has established a new space agency to make use of space research results in the national economy. The existence of the Main Administration for the Creation and Use of Space Technology for the National Economy and Scientific Research (*Glavkosmos*) was revealed last month in an unremarkable middle-page interview in *Izvestiya* with the new agency's head, A.I. Dunaev. This reticence is all the more remarkable because, when the Soviet press daily castigates President Reagan's strategic defense plans, *Glavkosmos* might have been used as evidence of the more peaceful Soviet approach to space.

Reticence, however, seems to be the keynote of *Glavkosmos*. The interview does not reveal when it was established, only that it "has been set up and has started working". Dunaev, however, refers to all its activities in the future tense, suggesting that it is a very recent innovation. Dunaev's own identity, too, remains obscure. Nothing is said of his career or academic qualifications, his background or even his given name and patronymic.

Even the purpose of *Glavkosmos* itself remains obscure. Dunaev notes the benefits of space research to the Soviet economy; long-distance communications (including the simultaneous printing of Moscow newspapers in Siberia and the Far East), meteorology, land-resources survey, navigation and the international "Kosmos-Sarsat" air-sea rescue system as well as the production of new superpure and biologically active materials aboard manned spacecraft. But his list reads like a

summary of the Soviet presentation at the United Nations conference on the peaceful uses of space (UNISPACE-82) three years ago.

Within the Soviet Union, the main purpose of *Glavkosmos* seems to be administrative. A number of ministries and government departments, Dunaev said, are concerned as are scientific organizations.

A special coordinating body is now needed, and *Glavkosmos* will have charge of the planning and space projects for the collection and dissemination of space information for practical purposes, and will also be responsible for Soviet participation in international cooperation programmes, both the Comecon "Interkosmos" project, and broader programmes such as the current *Veha* (Venus/Halley) probes in which nine countries are cooperating.

Whether *Glavkosmos* will be responsible for Soviet manned programmes is unclear. Dunaev's reference to future long-term stations is part of a general outline of space plans, not of the specific functions of his agency. Also unclear is the relationship of *Glavkosmos* with the Space Research Institute of the Soviet Academy of Sciences, which so far has acted as coordinator for the transmission of space data to the production sector.

Glavkosmos will not, it appears, be responsible for research planning, and its creation seems merely to interpose one more body between research and production. But it is precisely here that endemic delays in the transmission of results occur.

Vera Rich

Soviet computer education

Teething troubles galore

SOVIET plans to introduce computer studies in the senior forms of every school throughout the Soviet Union may be delayed by up to six years, according to the Uzbek historian, U. Mannanov, a member of the joint commission on information science and computer technology. Launched by a resolution of the Council of Ministers of the USSR and the Communist Party central committee in March, the plan was one of the first major decisions of the Gorbachev era, and was widely acclaimed as the next significant step in implementing the "scientific and technological revolution". Computer studies were already operating on a pilot scale in Soviet schools, but the essential feature of the new plan was its universality — every school was to have microcomputers, and every 15- and 16-year-old would learn how to use them.

Fifteen pairs of blue jeans and a personal computer.....?



From the beginning, there seemed to be some doubt as to whether the hardware would be ready in time. At the end of March, the director of the Institute of Teaching Content and Methods of the USSR Academy of Pedagogic Sciences, Professor Vadim Monakhov, admitted that the choice of computers to install would be determined by availability, not suitability. In the event, many schools failed to get their computers and, when the new term began in September, were informed that they would have to teach "theoretical" computer studies until the hardware arrived. At the same time, leading articles in *Pravda* hailed the introduction of universal computer studies as a major advance in the building of a truly science-orientated society, implying that the delay in installing the computers was a fairly short-term one. Mannanov's revelation of the likely length of delay was made only in *Pravda Vostoka* (Eastern Truth), a provincial newspaper not normally available in Moscow.

Another problem is a shortage of teachers able to teach computer studies. During the vacation, summer schools were organized for the physics and mathematics teachers who would be taking the new courses. According to Mannanov, of those attending the courses

in the Uzbek SSR, 92 per cent had previously never seen a computer.

Although the logistic problems of computer education are obviously worse in the Soviet Union, where editions of textbooks (and now of software packages) have to be envisaged in editions of 4 million, several Comecon countries seem to be experiencing similar difficulties. Poland, with barely a thousand computers in schools, has declared a moratorium in practical computer education for at least two years. Even so, it seems that a large number of pupils are already computer-literate, either from official youth club facilities or through access to "privately acquired" Western computers, of which there are thought to be between 25,000 and 60,000 in the country.

Czechoslovakia, on the other hand, in

spite of an admitted 40 per cent shortfall in deliveries of computers to secondary and vocational schools, has declared computer education one of the two major priorities for the current academic year (the other is peace studies). In Bulgaria, one of the main producers of computers in the Comecon bloc, not all schools even in the capital, Sofia, have their own computers, and some use facilities which properly belong to the higher education sector.

Only in Hungary does computer education seem to be running to schedule. The Hungarians, moreover, seem more aware than many of their Comecon partners of the broader applications of classroom computers, so that during his visit to Britain last month, the Hungarian minister of education and culture, Dr Bela Koepecsi, particularly requested a visit to a secondary modern school where computers are used not only in the mathematics and science classes but also in the humanities.

Vera Rich

Japanese psychiatry

Hospital reforms under way

Tokyo

THE first steps towards reform of Japan's mental hospitals were taken last week with the publication of a new set of guidelines by the Ministry of Health and Welfare. Criticism of the way hospitals are run has been mounting for some years but the ministry showed little reaction until visits from human rights organizations, including the International Committee of Jurists (see *Nature* 316, 571; 1985), stimulated international criticism.

The chief aim of the new guidelines is to give patients in mental hospitals the right to communicate with the outside world. The almost total isolation of mental patients has been seen as a major factor in allowing recent abuses to take place. At the Utsunomiya Hospital, where violence was regularly used against patients and two were alleged to have been beaten to death, patients tried to communicate with the outside world by launching paper aeroplanes. Earlier, an incident at an Osaka hospital where a patient was beaten to death by hospital staff came to light only through the chance discovery of a message from one of the patients. He had written the message on a piece of paper, wrapped it around a stone and thrown it out of the hospital grounds.

Three new provisions are made: public telephones will be installed in closed wards and patients will be free to use them as they wish; patients will be allowed to correspond with anyone they like, including lawyers and human rights groups; and staff will not be present when visitors are received, unless specifically invited. Furthermore, it is recommended that the telephone numbers of human rights organizations be freely displayed.

Critics are far from satisfied: they point

out that the new guidelines are just guidelines and fear they will not be obeyed. They also stress that loopholes exist: communication can be limited if required for medical treatment or protection of patients, while letters may be examined if there is any suspicion that patients might, for example, be trying to send drugs out of the hospital. The only counter to possible misuse of these clauses is that any restrictions placed on patients must be entered on their medical records.

What many groups pressing for reform would like to see are the kinds of changes requested by the International Commission of Jurists, chiefly the setting up of a completely independent review tribunal that would have the right to inspect mental hospitals and to which patients would have right of appeal. At present a patient may be committed to a mental hospital by the superintendent of a mental hospital once the guardian's consent has been obtained and patients have no rights to demand independent medical evaluation. This problem may be tackled in a reform of the Mental Health Act promised by the government for next year.

Whatever legal changes are made, there will remain the difficulties of effecting the changes in attitude that lie at the root of the problem. Although a reform movement exists, and a small number of "half-way houses" and workshops have been set up to help patients back into society, the mentally ill remain outcasts. A quarter of the patients in mental hospitals do not receive even one visit a year. And as many as half the patients could be released from the hospitals if their families were willing to have them back. It is these attitudes that will eventually have to be tackled.

Alun Anderson

Research in Ecuador

Universities are squeezed

Quito, Ecuador

THE Galapagos Islands, long a symbol of ecological diversity and evolutionary theory, also symbolize the relative strength in Ecuador of the biological sciences, blessed both by rich natural resources on their doorstep and by government support.

Dr Tjitte de Vries, director of the biology department at Quito's Catholic University, manifests the optimism born of sufficient funds. Each of the three government agencies that support research supports one of his projects (in the highlands, the tropical rain forest and the Galapagos). Moreover, his grant from the Canadian Wildlife Service to study the effect of pesticides on the peregrine falcon exemplifies the flow of foreign aid into biological research in Ecuador. "If a scientist has the initiative", says Dr de Vries, "the money is there", both for equipment and the high logistic costs of working in the rainforest.

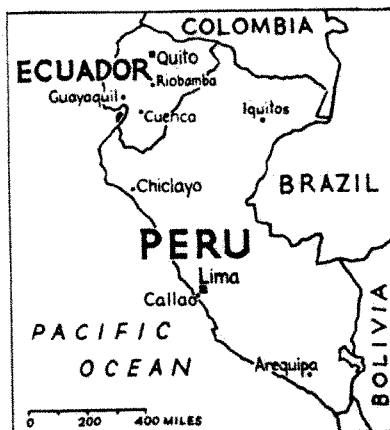
The most important governmental agency for university research is El Consejo Nacional de Universidades y Escuelas Politecnicas (CONUEP). Of the 7,000 million sucres (100 sucres=US\$1) distributed in 1985 from the Ministry of Finance to the 17 universities and polytechnics, 500 million sucres were assigned to research. Some research contract work is handled as well by El Consejo Nacional de Ciencia y Tecnologia (CONACYT), but research in the Galapagos is supported by the Charles Darwin Foundation, whose resources come from a mixture of government and foreign sources.

In 1984, CONUEP accepted 97 projects from 13 universities, out of 248 applications. There were as many applications in 1983, but only 25 were supported. Winning government aid depends on doing research in a priority area, as defined by El Consejo de Desarrollo (CONADE) in the National Development Plan 1984-88. Preference is given to projects in health and nutrition, agriculture and technology.

Researchers in Ecuador seem cheerfully to accept government-imposed directions for science. There is no secret about the priorities, which many feel are justified for Ecuador's development. For example, Dr Joan Llauger, head of the chemistry department at the Catholic University, shows no regret at leaving his speciality in metallurgy and concentrating on nutritional questions such as the oxidation of vegetable oil. The government has no history of giving funds to research, explained Mr Juan Maldonado, head of the Charles Darwin Foundation, so researchers have not yet come to consider such aid as their right rather than privilege.

Ninety per cent of government aid flows through the state universities, so the assets of the five private universities are particularly squeezed. Even in Dr de Vries' de-

partment, the highest degree offered is between a US bachelor's and master's. More infrastructure, says Dr de Vries, is necessary to reach international parity, primarily in libraries (the Catholic University cannot afford *Nature*), equipment, field stations and computer facilities. Fields dependent on expensive equipment, such as physics, are much harder hit. The physics department at the Catholic University only prepares teachers; it does no research. Dr Ernesto Cadena of that department says that even though a medical physics graduate programme has been started with foreign aid, there are not enough good physics teachers in high schools, no money for research laboratories, and few jobs for physicists in Ecuador.



Students interested in a higher degree are often encouraged to go abroad and then return to work. Dr de Vries, who is Dutch, came to Ecuador with UNESCO. Two members of his staff got doctorates in the United States, one in Germany, and another received a master's degree in Italy. Some of his students are on fellowship in Britain, the United States and Brazil.

The lack of tradition of research on an international level is the worst problem. Dr Llauger says he invites foreign professors to lecture and presents papers abroad every year, but that few of his colleagues (far fewer than in biology) do likewise. Few universities require publication from the professors.

The plethora of universities in such a small country is itself a doubtful boon. According to Dr Gonzalo Cevallos, who has taught economics at Quito's Central University and now works for the oil firm Intrade, the philosophy of free education has resulted in every town wanting a university. There are no entrance requirements, only a small percentage graduate, and few of these can find jobs.

"Ciencia aplicado" that augments the country's productivity is a clear government priority over basic research. Governmental entities perform much of this work, though, according to many, they are merely applying foreign techniques

rather than pursuing applied research. Funds from the state budget (for 1986, 201,000 million sucres) flow through the various ministries to institutes and government companies, which, says Dr Cevallos, do "research in brackets". INIAP, for example, is an institute under the Ministry of Agriculture that does plant research such as in cross breeding, and CEPE is an oil company controlled by the Ministry of Natural Resources and Energy. The military also has applied research arms: its Geographical Institute, for example, does topographical research, primarily mapping.

The techniques and equipment used by these institutes and companies, if not actually developed in Ecuador, are often the most advanced available. The Geographical Institute uses both US LANDSAT and French satellite facilities; the oil industry uses three-dimensional seismic technology; and the forestry department is importing a tissue culture laboratory to clone trees.

Oil and its byproducts are a primary source of funds for Ecuador's development. Because of its reliance on oil to support government projects and pay off foreign debt, Ecuador will not abide by OPEC's quota of 184,000 barrels/day and is instead nearing 300,000 barrels/day. Even in oil, says Dr Cevallos, it is more economic to use foreign techniques than to try to catch up to international levels of expertise.

Oil also helps to support the growing commitment in Ecuador to learning about the country's environment and how to use it wisely. According to Mr Maldonado, the government has accepted the foundation's advice on resource management, as evinced by an increase in support from an average of 70,000 sucres in 1964-66 to 10-15 million in 1966-85. Sixty wardens have been added to the national park system this year; the peasants are asking for reforestation projects; and a research station in the Amazon basin is studying how to raise alligators, fish and guinea pigs without destroying the forest. Ecuador and Costa Rica, says Dr Roque Sevilla, director of the National Forestry Programme, are the most advanced countries in this part of the world in terms of ecologically sound resource development.

Applied science is then, finally, paramount in Ecuador. That can be explained by the government's "practicality" and the need to spend money elsewhere.

There is another, darker possibility, however. Fear is spreading that the government, backed by banking and manufacturing interests, plans to strangle the universities, which are seen as catalysts of anti-government activity. One commentator says that recent increases in university research funds are nothing in the face of 35 per cent inflation: the government, a democracy only since 1979, "is out to crush the Left with no holds barred".

Elizabeth Collins

NERC studentships

SIR—Keith Runcorn (19 September, p.196) has obviously cogitated carefully over the report of the Natural Environmental Research Council (NERC) committee chaired by me which reviewed methods of allocating research studentships, since his letter appeared nearly a year after the report was published. His views are reminiscent of some of the evidence we received from heads of departments (possibly a slight majority), but which were opposed by a majority of other members of staff and by virtually all the students themselves. Our conclusions were not entirely maverick; we advertised our review as widely as we could and had about 400 comments from academics, employers, students and other research councils about the most equitable and positive ways of allocating studentships. Our recommendations were based on assessing all the information we had, giving appropriate weight to the relevance of the suggestions and criticisms.

It is of little general interest to respond to all the misapprehensions accumulated by Professor Runcorn. Perhaps the drafting of our report was obscure. But five points need making:

(1) The committee consisted of three Earth scientists, two former research council secretaries with backgrounds in the biological sciences (Sir John Gray of the Medical Research Council and Sir William Henderson of the then Agricultural Research Council, both of whom had overseen allocation procedures very different from that used by NERC), and myself, an ecological geneticist. We examined the past and present methods used by all five British research councils, and other possibilities suggested by our respondents. We were unanimous in our recommendations, and our report was endorsed by the NERC Council.

(2) Professor Runcorn opines that "the ratio of geophysical to geological studentships is too small both for scientific advance and for national needs". This is an appropriate and fashionable prejudice for a geophysicist to hold. The underlying problem is that it is extremely difficult to determine the proper allocation of studentships between disciplines. If demand is any guidance, requests in the NERC area for life science studentships are much greater than for Earth science ones. My committee recommended an "in-depth independent study with a view to laying down guidelines for the distribution of available awards between the (subject) committees", and this has been accepted by the NERC Council.

The general and very important problem of forecasting "national need" was analysed by a White Paper on Postgraduate Education (Cmd 6611, 1976) which expressed the view that "it is unlikely that a close match between supply and demand

could ever be achieved and it will be important to resist the temptation to forecast and plan with spurious precision". Sir Peter Swinnerton-Dyer's ABRC Working Party (1983) reviewed past endeavours on the subject and concluded that all these "attempts at manpower planning (for the supply of scientists and technologists) foundered principally on an uncritical faith in the economic contribution of education and on a confused concept of demand. . . since there are many PhDs whose working career has borne little relation to the subject of their thesis however potent the contribution of research training has been to their intellectual and practical development. It remains a truism that the quality of highly trained manpower is as significant as its quantity."

(3) Professor Runcorn repeats the accusation raised originally by the heads of university zoology departments that membership of a NERC committee improves the chance of getting a studentship project approved. If true, this would be worrying. The fact is that some committee members are more successful than expected in some years and less successful in others. There is no consistent picture, but (as Professor Runcorn notes) NERC "Council takes a very serious view (of this). . . important area which will be carefully monitored in the future". There need be no mystery or suspicion here: if the most active and productive research workers constitute the grants committees, they would be expected to obtain a higher than average proportion of awards. Perhaps the key factor is that the grants committees are not self-perpetuating cabals: for some time NERC has been inviting university departments and individuals to nominate to the committees, and this invitation was repeated in the committee's report. For the record, five of the 18 members of NERC's current geological sciences training awards committee are geophysicists.

(4) Professor Runcorn wants NERC to abandon its "effortful, cumbersome, expensive and unsatisfactory scheme". Ignoring his fourth adjective, our published response to this comment was that "as each research studentship costs in excess of £17,000 and represents three very important years of a student's life, the expenditure of a supervisor's time (in preparing an application) is fully justified".

(5) However, I suspect that the crucial difference between Professor Runcorn and my committee is over the purpose of a research studentship. We firmly and explicitly rejected the idea that a PhD student should be primarily a research assistant involved in an ongoing research project. Professor Runcorn quotes disparagingly "NERC's statement of the primary objective of a research studentship". Our understanding and conclusions were

based on two clear objectives. I am unrepentant about these:

"The primary aim of Council's policy must be to enable the intellectually most able graduates to receive the best possible training. An essential part of this training is the preparation of a PhD thesis, which also provides a means of ensuring that research undertaken is preserved and can be made available to others. Within the context of research training, it is council's aim to influence the quality of training and its direction and content.

"The second main objective of council's policy can be defined as ensuring that there is an adequate supply of manpower trained at postgraduate level to meet the national requirement in the sciences most closely concerned with the natural environment.

"A very important consequence of research training, even though incidental to that training, is the major contribution which research students make to the research output of universities. In itself, provision of training awards is a major contribution to the research community."

I am not clear why Professor Runcorn is so dismayed by our report. As a scientist, he cannot logically complain about an exercise that examines all relevant data and then excludes inadequate conclusions from it (even if he does not like the positive solutions that emerge). If it is any comfort to him, I was not allocated a NERC studentship this year, despite the fact that I put forward two *excellent* proposals to the grants committees. . .

R.J. BERRY

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Human life

SIR—The distinction between dependently viable protoplasmic life and independently viable organismal life (respectively at the level scale of the molecule and of the individual), as made by R. C. Hoult (*Nature* 8 August, p.480), is quite correct, although it does not follow that the life quality necessarily attributable to a fetus *in utero* is that of protoplasmic life only.

The fetus has undoubtedly an organismal life, and we can speak of "fetal behavioural states" (*Prenatal Diag.* July 1985, p.269) in spite of its dependence on its mother for energy supply. An infant seems not to differ very much in this respect. It must be recalled that, up to the onset of implantation, the human conceptus is "free living" in the sense that it is not attached to the mother in any way. Nor can I see how a human adult individual during parenteral nutrition could lose his "human life" or organismal quality.

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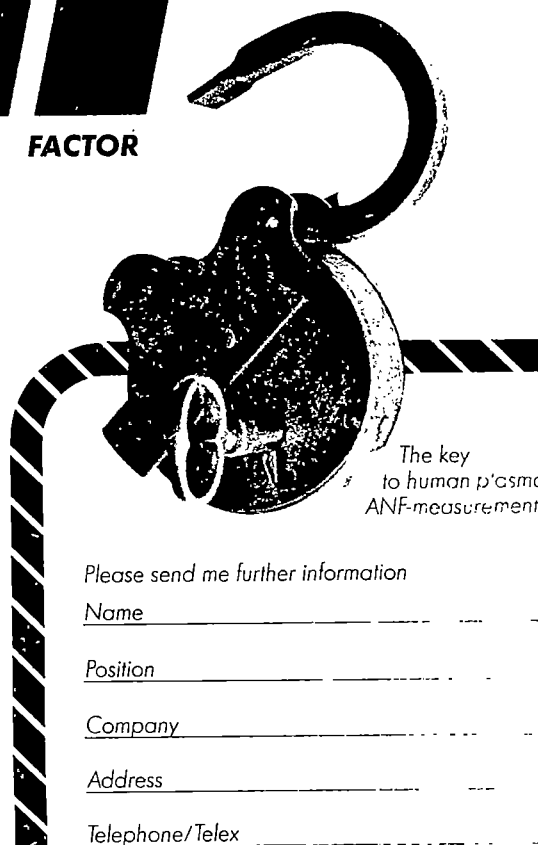
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The virtues of single atoms

Success in cooling single atoms to millikelvin temperatures, and then of trapping them for seconds (or even hours) on end, will benefit not merely spectroscopists but the rest of physics.

THE obvious benefits of being able to study single ions or even atoms are those that the spectroscopists have been extolling for several years, ever since it seemed probable that finely tuned lasers might be used to rob moving atoms of velocity in a specific fashion. Earlier this year, there was a flurry of excitement with the first demonstration that sodium atoms can be "cooled", or robbed of velocity, by the selective absorption of photons (and thus momentum) of a suitable frequency. (The technique and the consequences were fully discussed by B.W. Petley in a *News and Views* article on 27 June this year, p.716.)

Especially because it is now possible to trap electrically charged particles in suitably configured static electric and magnetic fields, the spectroscopists have had plenty to look for, not least the chance to trap small numbers of ions in excited states so long-lasting that the radiation they emit is neither made fuzzy by the Doppler effect, a consequence of movement, nor broadened substantially by quantum considerations, Heisenberg's uncertainty principle in particular. The notion that suitably prepared trapped atoms may make more convenient standards of frequency (or of time) than the more familiar atomic clocks of caesium is not entirely out of court.

But even before these opportunities have been exploited, the hunt seems well under way for a technique for trapping neutral atoms and molecules in apparently empty space. The first informal report of success in this direction came from Harold Metcalf of the State University of New York at Stony Brook (also in *Nature* on 27 June, p.717) hinting at experiments by his own group and by another at Bell Laboratories in which neutral sodium atoms had been successfully trapped for periods of time measured in seconds. Now it seems that more systematic ways of achieving these objectives are in sight.

The article by R.V.E. Lovelace, C. Mehanian, T.J. Tommila and D.M. Lee, all of them from various parts of the physics enterprise at Cornell University, is not so much the report of a discovery as a challenge to other experimenters. They explain that the goal they have set themselves theoretically is to define the circumstances in which it would be possible to trap atoms of neutral hydrogen and then to explore the question whether such an assemblage could ever be made dense and cool enough so as to undergo the

Bose-Einstein phase transition expected of all bosons, particles (in this case atoms) with integer spin (zero included).

Will the challenge be taken up? A glance at Table 1 on p.31 will show how formidable a task lies ahead. At 0.1 degrees above absolute zero (or at 0.1 K), it would be necessary to compress hydrogen atoms dealt with singly to roughly the density expected from molecular hydrogen gas at normal temperature and pressure. Doing this in a vacuum will require a substantial magnetic bottle. But how can such a device, familiar enough in the manipulation of some plasmas which are candidates for thermonuclear fuel, be made to function for atoms which have no electric charge? The clever trick that Lovelace *et al.* describe entails exploiting the potentially different orientations in a magnetic field of the nuclear and electron spins of single hydrogen atoms. It should be possible to trap those atomic arrangements in which both the nuclear and electron spins are parallel to an external magnetic field by exploiting their tendency to congregate at the places where the field is greatest. The snag is that collisions between atoms would require that the experiment would be feasible only at a temperature of 0.0001 K, thought still to be unattainable. Whence the neat proposal that an oscillating magnetic field should be superimposed on a static field in such a way as to create an oscillating magnetic bottle to pump atoms with different hyperfine structure to different regions.

It remains to be seen whether the scheme proposed by Lovelace *et al.* can be exploited as they suggest, but the fact that the scheme has arisen at this stage is a proof of how far the physics of single atoms has developed in the past year or so. Whatever may be the defects of this scheme, nobody would have thought it sensible to put it forward five years ago. And it is also plain that the interest of schemes for trapping single atoms and molecules, or small numbers of them, extends far beyond spectroscopy. B.W. Petley explained in June how measurements of stable confined atomic systems could be used to test the isotropy of gravitational inertia. T. Erber and S. Putterman, on p.41 of this issue, raise another set of questions in fundamental physics that may be answerable by means of atoms and molecules confined in traps. Can such a system be used to test the randomness of the physical processes such as the decay of the

excited state of an atom?

In principle, it might be possible to carry out such a study by means of a single atom or ion that is suitably trapped in a potential well, using some kind of radiation source to excite it and a suitable photomultiplier to detect the photons arising from radiative decay. But the photons used for excitation would have precisely the same frequency as those to be detected by the photomultiplier. If the average lifetime of the excited atom were to be large enough for the intervals between excitation and decay to be measurable, the excitation would be only weakly coupled to the exciting radiation, which would enhance the likelihood that the photomultiplier would be more often activated by exciting radiation than by that from a genuine decay of an excited atom.

So Erber and Putterman propose a neat atomic switch, a way of telling whether a single atom is at any point excited into one of these comparatively long-lived states. To fit the need, the atom must have two excitation levels, one of which is short-lived and the other long-lived. By means of two lasers, one tuned to each of the excitation frequencies, it should be possible to be sure that the atom at any time is in one of three states, the unexcited ground state or one or other of the two excited states. But the amount of time for which the atom is in the short-lived state can be measured by recording the photons arising by the spontaneous fluorescent decay of that short-lived state. Moreover, because the frequency of the transitions in each direction will be high, if the disparity of lifetimes is great enough, when there is no short-lived fluorescence, the atom should be excited into the long-lived state. In other words, the experiment that naturally suggests itself is a means of measuring the length of time for which a single atom persists in the long-lived state.

Erber and Putterman go on to say that these lifetimes, which should be unaffected by any influence of the external observer, should be strictly random numbers. Paradoxically, they remark, the simplest of all physical systems, a single atom caught in a trap, should be more capable than all but the most complicated computing machinery of generating strictly random numbers. The more important point is that the system is yet another way of testing predictions of quantum mechanics. That is another challenge, but increasingly an academic one.

John Maddox

Developmental biology

Mice, mating types and molecular mechanisms of morphogenesis

from Miranda Robertson

Two meetings this year* have marked the arrival of a molecular biology of embryonic development. At both, much emphasis has been placed on the universality of developmental mechanisms in phylogeny. In what follows, I shall use a few selected examples to illustrate the parallels that are being drawn between mice, flies and microbes and to examine briefly what they may mean.

I should state at the outset of this article that the omission of *Drosophila* from its title is not merely a matter of assonance: the dazzling achievements of *Drosophila* molecular genetics have been extensively discussed in earlier articles in these pages and the most recent advances are soon to be the subject of another. I shall therefore mention them only in connection with the arresting discovery that the so-called homoeo-box sequences, comprising part of the homoeotic genes that control *Drosophila* morphogenesis, have highly conserved counterparts in vertebrates (see ref. 1). The hope has been that these sequence homologies may provide one of two new routes to the genes that control morphogenesis in mice and men. The second route that has opened up at much the same time is through the transgenic mouse. DNA microinjected into a mouse embryo will occasionally become integrated into its chromosomes in such a way as to disrupt the normal process of development and thus in principle act as a traceable marker for a gene involved in its control. The work reported by Woychik *et al.* in this issue of *Nature*² has already been publicized³ as a success for the second approach: in a series of experiments in Philip Leder's laboratory in which the *c-myc* gene was injected into mouse embryos, on one occasion the gene fortuitously became integrated so as to produce a recessively inherited deformity of the limbs.

How far can the deformed mouse be regarded as a mammalian equivalent to the homoeotic mutants of *Drosophila*? And how likely is it that the mammalian homoeo boxes will provide a direct route to genes essential for morphogenesis in mammals?

Mice

The homoeo boxes of *Drosophila* are coding sequences of DNA, usually about 180 base pairs in length, first discovered at the 3'-end of the homoeotic genes, mutations in which cause the transformation of one

body part into another. Homologous sequences are found in worms, frogs, mice and man — and in yeast, which seems an embarrassment to their putative role in metazoan morphogenesis (but see later).

Drosophila homoeo boxes fall into two classes (1 and 2), which are about 50 per cent homologous in sequence. But the homology between the homoeo boxes of each class across species is about 80 per cent. At the time of its discovery this was, and is still, the most compelling evidence that the sequence homologies between the homoeo boxes of fruitflies and vertebrates have any functional significance. Other kinds of evidence are somewhat thin. It is now clear that these sequences are expressed in vertebrate embryos, and that their expression is in some cases temporally regulated and tissue specific. Class 1 sequences, for example, are expressed from day 13 in mouse, principally in the dorsal neural tube (Frank Ruddle, Yale); Gail Martin (University of California, San Francisco) finds expression of both class 1 and class 2 sequences between about the ninth and seventeenth days of gestation, and in embryonal carcinoma cells of mouse⁴ and man (Robert Tjian, University of California, Berkeley) class 1 sequences are expressed only after the induction of differentiation.

There is, however, nothing to link any of this with morphogenesis. Nor do mouse genetics provide evidence of anything that might be a homoeotic mutation. There is a morphogenetic mutant (*Tail-short*) that maps near the class 1 homoeo-box cluster on chromosome 11 (see ref. 1); but the association may be entirely adventitious. The mutation induced by *c-myc* in the transgenic mice of Woychik *et al.* may prove more informative.

The principal feature of the limb deformity in the mutant mice is the fusion of some of the distal bones of the limbs, and Leder and his colleagues have been able to show, through genetic crosses, that the mutation is allelic to a natural recessive mouse mutation, *ld*, that also causes bone fusion and maps, like the inserted *myc* gene, to chromosome 2. There is, moreover, a naturally occurring dominant mutation, *Isr*, that maps to roughly the same region and causes the reverse phenotype — bone duplication. The dominant mutation has not yet been shown to be allelic to the recessive; but if it is, the two mutations may reflect, respectively, an excess and a deficiency of a morphogen or growth factor⁵. In principle, then, analysis of the site of the *myc* insertion, which has already been cloned,

should yield the intact gene encoding the factor, and the first identification of an embryonic morphogen.

In practice, however, it has not yet been possible to identify the inactivated gene and Leder's optimism is somewhat guarded, as was the response to his presentation at Cold Spring Harbor. Morphogenetic mutants are not always interesting, as experience with invertebrates has shown. There is for example a *Drosophila* wing-deformity mutant, *rudimentary*, that is simply the consequence of a metabolic deficiency affecting pyrimidine synthesis; and it was repeatedly pointed out that quite non-specific perturbations may disrupt development in an apparently systematic way (this has been a problem, for example, with attempts to apply to vertebrates the technique of specific inhibition of gene expression with anti-sense RNA). It seems unlikely that the fruitfly fraternity will be impressed by mouse geneticists until they can produce a mutant with a perfectly formed paw whose only abnormality is that it ought to have been an ear.

Mating types and mechanisms

In the absence of any evidence of a role for the vertebrate homoeo boxes in the genetic control of development, what might the evolutionary conservation of the homoeo boxes signify? An answer may be suggested by the ostensibly surprising discovery of homologous sequences in yeast — though it is only fair to say that the homology between yeast and metazoan homoeo boxes is of an altogether lower order than those between the metazoan sequences.

It is believed that *Drosophila* homoeo boxes encode protein domains that bind specifically to DNA. It is therefore probably significant that the yeast sequences homologous to the *Drosophila* homoeo box occur in the gene encoding $\alpha 2$ protein, a regulatory molecule that coordinates the expression of genes determining the mating type of yeast cells by binding to a specific sequence in their flanking DNA⁶. It has long been the guiding principle of Ira Herskowitz's laboratory, in which most of the investigations of the $\alpha 2$ protein have been performed, that the control of mating type in the single-celled yeast embodies the fundamental principles of differentiation in higher animal cells. Moreover, yeast mating type has one striking advantage over fruitfly homoeosis: while nothing is yet known about the molecular basis for the homoeotic phenotype, a good deal is now known about the molecular mechanisms that underlie the specification of yeast cell type.

The yeast *Saccharomyces cerevisiae* has two haploid mating types, *a* and α , each of which releases a factor that binds to a specific receptor on cells of the opposite mating type. This causes the two cells to fuse to form an a/α diploid. In *a* cells, the genes specifying the α factor and the *a*-

*The Cold Spring Harbor Symposium on Molecular Biology of Development, 29 May–5 June 1985, and *Nature's* Conference on Genes and Systems in Development, San Francisco, 6–9 October 1985.

factor receptors are silent, whereas those specifying the a factor and the α -factor receptor are active; the converse is the case in α cells; and in a/α cells, both sets of mating-type genes are silent and those that control sporulation, which are silent in the haploids, are activated. Mating type is controlled by a single locus, the mating-type locus *MAT*, which contains an a allele in a cells and an α allele in α cells. The gene products of these alleles are regulatory proteins. Herskowitz and his colleagues have been able to show that $\alpha 2$, one of two products of the α allele, binds to a specific sequence upstream of the a -specific genes and represses them (refs 5 and 6, and Kathy Wilson, University of California, San Diego). The other product, $\alpha 1$ activates the α -specific genes. In a cells, the α -specific genes are silent because of the absence of $\alpha 1$, and the a -specific genes are active because of the absence of $\alpha 2$.

In a/α cells, a completely new pattern of gene expression is generated by the combination of regulatory proteins from both *MAT* alleles. The most recent evidence from Herskowitz's laboratory suggest that the sporulation genes are activated in the diploid cell by repression of *RME1*, a gene whose product inhibits sporulation genes in haploids. The repression of *RME1* requires $\alpha 2$ and $\alpha 1$, which is a product of the *MAT a* allele. Although its mechanism is unknown, the DNA-binding properties of $\alpha 2$ make it likely that an $\alpha 2 - \alpha 1$ complex binds, perhaps cooperatively, to a regulatory site in the *RME1*-flanking DNA. Thus the combination of two regulatory proteins may generate a novel specificity not possessed by either alone. This proposition has been

strengthened by the identification of a presumptive $\alpha 1 - \alpha 2$ binding site whose sequence is related to the known $\alpha 2$ binding site⁷.

Back to mice

How does the molecular mechanism of mating-type determination in yeast help with the two questions with which I began: what is the significance of the homoeo-box homologues, and can transgenic mice provide a mammalian alternative to the homoeotic mutants of fruit-flies? It is not known whether the homoeo-box encoded domain of $\alpha 2$ is directly involved in the binding of DNA, but it is known that mutations in this region abrogate its activity. Since its activity has been shown⁸ to depend upon the formation of $\alpha 2$ multimers as well as on binding to DNA, the homoeo-box encoded domain may be responsible either for binding DNA or for binding between the protein monomers,

It follows that if the conserved homoeo-box sequences do, as seems likely, reflect conserved functions, then it is their binding specificity that is being conserved; but it does not necessarily follow that this binding — whether to DNA or to other proteins — has anything to do with the control of development. There is no dearth of evolutionary precedents for the use of the same highly conserved structure in different physiological pathways: yeast and mammalian *ras* have recently turned out to be a case in point (see ref. 8) and it is a commonplace that a highly conserved hormone may act through a highly conserved receptor to produce a wide variety of unrelated effects in different cells.

What the yeast data may illuminate are the principles on which gene regulation

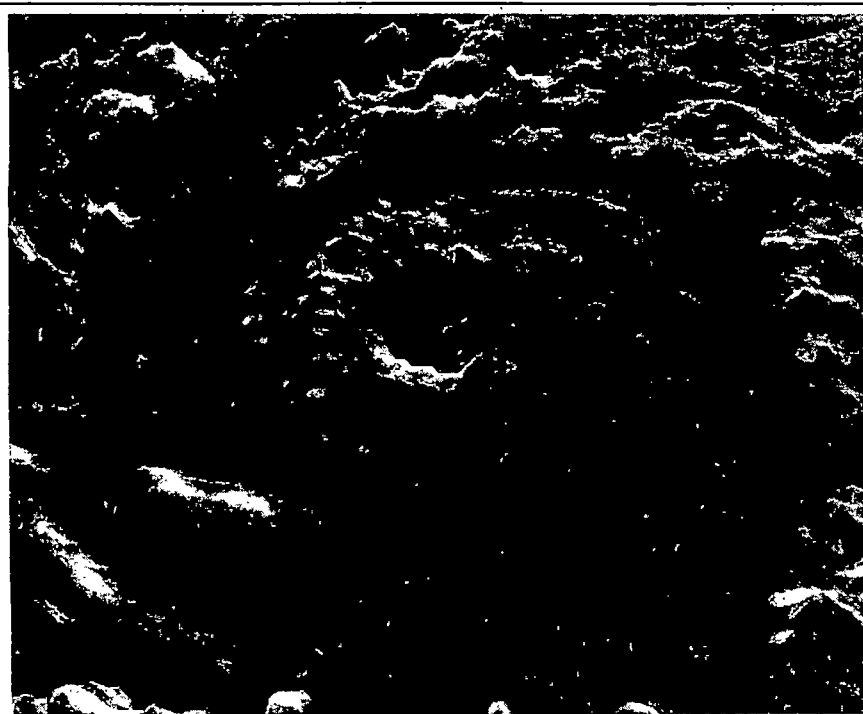
may work during embryogenesis, rather than the actual proteins through which a particular pattern of regulation is brought about. For example, it is generally accepted that differential gene regulation during development must be controlled in part by signals between cells. Thus it must depend on the temporally and spatially patterned expression by developing cells of the genes encoding the signals and their receptors. Some patterning of this sort is present in microcosm in yeast: in some strains, the a and α mating types (and thus the two factors and their receptors) are expressed in alternate haploid generations, but with the complication that only mother cells may make the switch. The mechanism of this patterned switching is not fully understood but analogous mechanisms may underline patterned developmental switching in higher organisms.

And while changes in gene expression in embryos are not likely to be brought about by the fusion of cells of two different types, it is extremely likely that differentiation is the consequence of varying combinations of cooperating regulatory proteins, as with $\alpha 1$ and $\alpha 2$ of yeast (induction of a new regulatory gene by an extracellular signal could take the place of the fusion of haploid cells in producing new combinations). As it happens, a transgenic mouse recently reported in *Nature*⁹ may provide an approach to the question of novel regulatory combinations in mammalian development. Swanson *et al.* have produced a mouse containing the growth-hormone gene fused to the metallothionein promoter and expressing the fusion gene in selected subsets of neurones in the brain, where neither growth hormone nor metallothionein is normally expressed.

It is traditional in development biology to distinguish between differentiation and morphogenesis. Under this distinction, it is differentiation that is disrupted in the Swanson *et al.* transgenic mouse, and morphogenesis in the Woychik *et al.* mouse. At the molecular level, however, differentiation and morphogenesis have the same mechanism, morphogenesis simply being a consequence of differential expression of genes that control developmental switches. It is the power of the yeast system to show how differential gene expression at a master regulatory locus, such as *MAT*, may give rise to patterns of differential gene expression of the sort that must underlie morphogenesis. □

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Miranda Robertson is Biology Editor of *Nature*.



Hurricane Allen on 8 August 1980 when its eye was above the Gulf of Mexico, as shown by application of a new computer imaging technique to meteorological data (Hasler, A.F., Pierce, H., Morris, K.R. & Dodge, J. *Bulletin of the American Meteorological Society* 66, 798; 1985).

Angiogenesis

Isolation of a protein that stimulates blood vessel growth

from Lance Liotta

A TEN-YEAR scientific quest to characterize a substance that can stimulate angiogenesis — the growth of new blood vessels — has ended with a triplet of papers in which Bert Vallee and colleagues describe the purification of a protein they call angiogenin and the cloning of its gene¹⁻³. The quest was based on the pioneering work of Judah Folkman who demonstrated that tumour growth requires nourishment by the continual ingrowth of new blood vessels, and suggested that this is the result of a diffusible substance that he termed tumour angiogenesis factor (TAF)^{4,5}. Recognizing the therapeutic potential of an angiogenesis inhibitor, the Monsanto Company provided a \$23 million endowment to Harvard University in 1976 to support Folkman with Vallee, who was to contribute the biochemical expertise necessary for rigorous protein purification. About five years into the research, Vallee and Folkman split their research into independent directions. As a result, Folkman is not associated with the papers on angiogenin, which Vallee considers to be different from TAF.

Folkman and Vallee have used different approaches to the purification of angiogenic substances and this has led to the discovery of two types of purified substance. One is exemplified by a heparin-binding growth factor identified by Folkman's group. Folkman reasoned that angiogenesis begins with the migration and growth of capillary endothelial cells. His group therefore developed the first cloned cell lines of capillary endothelium, and *in vitro* assays to identify and purify factors that are mitogenic for endothelial cells. Klagsbrun and Shing in Folkman's laboratory purified a heparin-binding endothelial mitogen which induces new vessel formation when injected into the rabbit cornea⁶. Subsequently, several groups purified heparin-binding endothelial cell mitogens that are angiogenic⁷⁻⁹. A recent example is the report by Gospodarowicz's group⁷ of two polypeptide mitogens for endothelial cells. The primary structure of one of them — bovine pituitary basic fibroblast growth factor (FGF) — was determined and compared with the amino-terminal sequence of the other — brain acidic FGF. The purified basic FGF is a potent angiogenic substance in chick chorioallantoic membrane (CAM) assays.

Instead of using the endothelial cell as a starting point for purification, Vallee's group has accumulated and used large quantities (2,000 litres) of conditioned medium from human colon carcinoma cultures. The proteins in the medium were

fractionated by cation exchange and reverse phase liquid chromatography, and directly assayed for angiogenesis in the CAM assay. Purified angiogenin has a relative molecular mass of 14,000 and does not bind heparin. Only 3.5 pmol are required to induce blood vessels in the cornea by a mechanism that is apparently unrelated to endothelial migration or proliferation.

It is widely believed that angiogenin and the heparin-binding proteins may be members of a whole family of factors that induce and regulate the growth of new blood vessels; the angiogenesis literature contains many reports concerning a variety of different factors at various stages of purity, all of which can induce vessel growth in the cornea. From one point of view this is expected, since the development of a new capillary network is a complex process involving a number of steps. One of the first steps may be dissolution of the subendothelial basement membrane at the point of endothelial cell outgrowth¹⁰. Subsequent steps include endothelial locomotion, endothelial tube formation, development of capillary loops, renewed establishment of the basement membrane and maturation of the vascular channels^{5,11}. These steps may be coordinated by multiple signals from the endothelium and other host cells in response to the angiogenic stimulus. Angiogenesis may be analogous to the blood coagulation cascade in that one event leads to the next through the action of specific mediators. Although

angiogenin is not the only substance that can induce angiogenesis, it may be a key trigger for the process.

The most significant accomplishment of Vallee's group is the cloning of the angiogenin gene, which turns out to be one of the small number of mammalian genes known that lacks introns. The amino-acid sequence of angiogenin predicted from the gene sequence is strikingly homologous to human ribonuclease, including conservation of the amino-acid residues that are essential for the activity of ribonuclease. The physiological significance of the homology is unclear, since angiogenin lacks ribonuclease activity and ribonuclease itself is not angiogenic. Expression of the gene for angiogenin is probably not limited to tumour cells. Nevertheless, angiogenesis may be more tightly regulated in normal tissues than in neoplastic tissues.

The cloning of the gene for angiogenin now makes it possible to study one potential mode of regulation of angiogenesis. The gene can also be used to produce large quantities of angiogenin so that its mechanism of action can be fully studied. This may ultimately lead to pharmacological strategies for inhibiting or augmenting the growth of blood vessels. Control of angiogenesis could have an impact on human diseases ranging from neoplasia to myocardial ischaemia. □

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Palaeontology

Fossil radiography

from Simon Conway Morris

MUSEUM visitors will be familiar with the striking brassy-yellow colouration of many ammonites, the result of replacement of the fossil by iron pyrites (FeS₂). Not surprisingly, this replacement typically involves the hard skeletal parts that provide the stock-in-trade of most palaeontologists. There are, however, extraordinary instances where pyritization may preserve the soft parts and more lightly skeletalized components of animals and plants from the otherwise inexorable processes of decay (Fig. 1). The Lower Devonian Hunsrück Slate (about 400 Myr) of West Germany is widely recognized as one of the most important deposits for soft-part preservation in the fossil record, and on page 53 of this issue

Wilhelm Stürmer describes another major find from this palaeontological gold-mine in the form of a unique specimen of a squid, aptly named *Eoteuthis*.

Stürmer's description is important on several counts. Not only does this discovery extend significantly the geological timescale of the teuthids, but it may ultimately result in a revision of current notions about cephalopod phylogeny, especially for the coleoids (squid, cuttlefish and octopus). At present, because coleoids generally lack preservable hard-parts, there is a lamentable fossil record that is almost entirely derived from Jurassic or younger rocks, and there is little consensus concerning the phylogenetic relationships within the squids and their

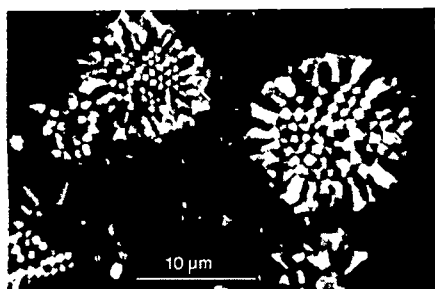


Fig. 1 A back-scattered electron image obtained with a scanning electron microscope of pyrite framboids from exceptionally well preserved trilobites with soft parts, from the Beecher's Trilobite Bed, New York State.

relatives.

Eoteuthis is one more addition to the roster of remarkably diverse finds from the Hunsrück, which boasts a wealth of otherwise poorly known forms. Among others, there are trilobites with preserved appendages, pycnogonids and a variety of more enigmatic arthropods, ctenophores, polychaetes, nautiloids, starfish and crinoids (Stürmer, W., Schaarschmidt, F. and Mittmeyer, H.-G., *Kleine Senckenberg-Reihe* 11; 1980). This wealth of fossils, providing the most complete insight into a mid-Palaeozoic marine community, continues to be augmented both by amateur collectors and from Stürmer's private quarry in Kaisergrube.

Stürmer uses sophisticated X-ray equipment and high-resolution photographic materials to produce radiographs of pyritized fossils concealed in the slate (Fig. 2). The density of the pyrite in contrast to the surrounding shale makes radiography particularly suitable, especially if the specimen is largely concealed with the rock. By carefully controlling the conditions of exposure and using image enhancement, formerly obscure structures may be revealed to a resolution of at least 5 μm . His recent descriptions of the Hunsrück biota and of material from other special palaeontological sites (Stürmer,



Fig. 2 Radiograph of a fossil crinoid from the Hunsrück Slate. Also known as sea lilies, crinoids are echinoderms, related to starfish and sea urchins. Only two specimens of this species have been found and this picture has been assembled from radiographs of several species. (Photograph courtesy of W. Stürmer).

W. *Interdisc. Sci. Rev.* 9, 1; 1984) highlight the success of the techniques. They contrast with traditional methods of preparing Hunsrück fossils by mechanical excavation that require utmost care to avoid damaging the gossamer films of pyrite.

The precise conditions governing pyritization in the Hunsrück are still obscure. The preservation of soft-parts, probably by filling cavities or forming coatings, by pyrite precursors such as greigite (Fe_3S_4), probably took place very shortly after burial. This would accord with recent textural and sulphur isotope studies of pyrite paragenesis (Hudson, J.D. *Sedimentology* 29, 639; 1982 and Love, L.G., Coleman, M.L. & Curtis, C.D. *Trans. R. Soc. Edinb. Earth Sci.* 74, 165; 1983), and suggests that this early stage of pyritization could persist only as long as bacterially

mediated sulphate reduction occurred. Interruption of sulphate influx from the overlying seawater, perhaps by rapid sediment deposition, could have arrested the bacterial activity that was presumably contributing to the overall biodegradation. But this hardly explains the selective replacement of the Hunsrück organisms, or the scarcity of similar fossil occurrences. Another example is the celebrated Beecher's Trilobite Bed in the Upper Ordovician of New York (Cisne, J.N. *Palaeontograph. Am.* 9 (53); 1981). Perhaps the rarity is more apparent than real and surveys of other fine-grained sediments will yield some surprises.

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Nuclear physics

Pions still pose problems

from Philip J. Siemens

A REPORT¹ that pi mesons, or pions, have been produced in collisions of complex atomic nuclei accelerated to only 23 per cent of the speed of light has added to the problems of nuclear physicists trying to explain pion production in low velocity nuclear collisions. Simple predictions based on the idea that pions result from collisions between individual pairs of neutrons or protons fail to account for pion production at such low collision velocities and the hope is that the phenomenon will reveal new details of nuclear structure.

Since each nucleus will normally exist in its state of lowest energy, a pion cannot be removed without supplying sufficient energy to provide not only for the pion's kinetic energy of motion, but also for the energy mc^2 associated with its mass m . The same amount of energy would also be sufficient to create a new pion that had not been part of the original nucleus.

The more usual way of liberating pions from nuclei (or creating them — the two processes cannot be distinguished) is to bombard nuclei with accelerated projectiles such as neutrons or protons; the kinetic energy of the accelerated particle produces or liberates a pion when it strikes the nucleus. Starting with a knowledge of how pions are produced in collisions between individual neutrons or protons, theorists have been able successfully to predict features of the production of pions from complex nuclei, such as how often they are produced and their distribution in energy and angle. These predictions are based on the idea that a pion is created when the projectile — which may be a photon, electron or pion, as well as a neutron or proton — strikes one of the neutrons or protons in the target nucleus; the resulting pion may either escape or be reabsorbed by the nucleus.

The difficulties start when complex nuclei are used for projectiles as well as for targets. In the past few years, new accelerators have been commissioned that are able to accelerate complex nuclei, such as carbon or neon, to speeds where the total kinetic energy of the nucleus is sufficient to create a pion, whereas the kinetic energies of the individual neutrons and protons are too small for the purpose. Although pions are seldom produced in these collisions — as rarely as once in a hundred million collisions — they have been observed, initially in 1979 at the Lawrence Laboratory in Berkeley, California². Surprisingly, the pions were produced from beams of nuclei with a velocity of 0.43 c , whereas the minimum velocity necessary to produce a pion from a neutron or proton striking a target of protons is 0.64 c . In 1982, an experiment³ at CERN in Geneva produced pions from nuclear beams with a velocity of 0.4 c and the following year⁴ the National Superconducting Cyclotron Laboratory in East Lansing, Michigan yielded pions from beams of a velocity of only 0.27 c . This downward trend is continued by the 0.23 c reported by Oak Ridge National Laboratory in Tennessee⁵. Experiments in progress at accelerators at Caen, France and at Texas A&M University in the United States may continue the trend.

Because the pions observed in these experiments cannot be produced by collisions of individual pairs of neutrons or protons, they ought to reveal information about the structure of the nucleus as a whole. Two theoretical models have been partially successful in interpreting the observations. The first model, by Jorg Aichelin and George Bertsch of Michigan State University⁶, explains the number of pions by assuming that the projectile and

target fuse together, distributing the projectile's kinetic energy throughout the combined nucleus in the form of heat. Applying statistical mechanics, this model gives a good account of the pions' energies for the faster beams but cannot explain why their average kinetic energy does not decrease as the beam velocity falls below 0.35 *c*. Below this speed, the pions' kinetic energy distribution seems to retain a high temperature, even though the excited nuclei that emit them are cooler. The model also makes no prediction about the angles at which the pions are emitted.

To explain the angular distributions of the pions requires quantum mechanics, because the wavelength of their matter waves is larger than the size of the nucleus that emits them. So far, the only applicable quantum theory has been developed by Walter Greiner, Berndt Mueller and co-workers at the University of Frankfurt. This model draws on an analogy with the emission of electromagnetic waves: the deceleration of the nuclei as they collide provides a time-varying current which causes radiation of pionic matter-waves. This theory gives a reasonable account of the angular distribution⁶ but predicts —

contrary to experiment — that no pions will be emitted in collisions of carbon beams with carbon targets, since carbon has zero isotopic charge (the pionic analogue of the electric charge). An *ad hoc* modification of the theory would permit a reasonable reproduction of the data but would fail at still lower energies, where it would continue to predict pion emission even when the total energy is insufficient.

The theorists' inability to understand the cooperative mechanism of pion production in low-velocity nuclear collisions has not discouraged the experimenters from competing to lower the velocity even further. Since pions are the main agents of the strong forces that hold nuclei together, theorists hope that the data will eventually provide a better understanding of nuclear forces. □

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Geology

East Asian tectonic collage

from A.M.C. Şengör

As the accompanying map shows, Asia consists of numerous continental and oceanic fragments stitched together by continental collisions. These not only enlarged the continent, but also disrupted parts of it through collision-related rifting and strike-slip faulting, as may be seen in the current convergence between India and Eurasia¹. During the last decade China has emerged as an ideal place to study the processes of continental collision and evolution of orogenic collages²⁻⁶, offering a tremendous variety of collisional mountain belts that are now in, or were fossilized at, different stages of their evolution. Two papers in a recent issue of *Nature*, by Mattauer *et al.*⁷ and Peltzer *et al.*⁸ deal with the tectonics of one of China's most important mountain ranges, the Qin-Ling Shan — "the backbone of China proper"⁹.

Since the time of the expeditions of P. Armand David in 1866, Count Bela Széchenyi from 1877 to 1880, and especially Baron Ferdinand von Richthofen from 1868 to 1872, the Qin-Ling Shan has attracted the attention of geologists. The unravelling of how and when eastern Asia was assembled is critically dependent on the tectonic evolution and timing of collisions along this belt, but even the establishment of the age of the main deformation has been no easy matter. Mainly on the basis of von Richthofen's observations, Suess¹⁰ described it as a orogenic belt of medial Carboniferous age, whilst

Argand¹¹ saw in it a 'Hercynian chain' with pre-Devonian enclaves. In the first detailed study of the chain in 1929, Chai and Huang⁹ revised von Richthofen's stratigraphy and moved the age of the main orogeny into the Jurassic. Later, in his now-classic 1945 memoir¹², T.K. Huang pointed out that recent research had established the age of the Qin-Ling Shan as "pre-Jurassic". Although Huang left open the possibility of a late Palaeozoic ('Hercynian' as he called it) deformation, he strongly favoured an Indonesian (late Triassic) orogeny. During the last two decades, opinions on the age of the main orogeny along the Qin-Ling Shan have varied from Devonian (400 Myr) to late Triassic (210 Myr), with the majority of the Chinese and foreign authors favouring an Indonesian orogen with a polycyclic history stretching through the 'Caledonian' and 'Hercynian' cycles¹³⁻¹⁸.

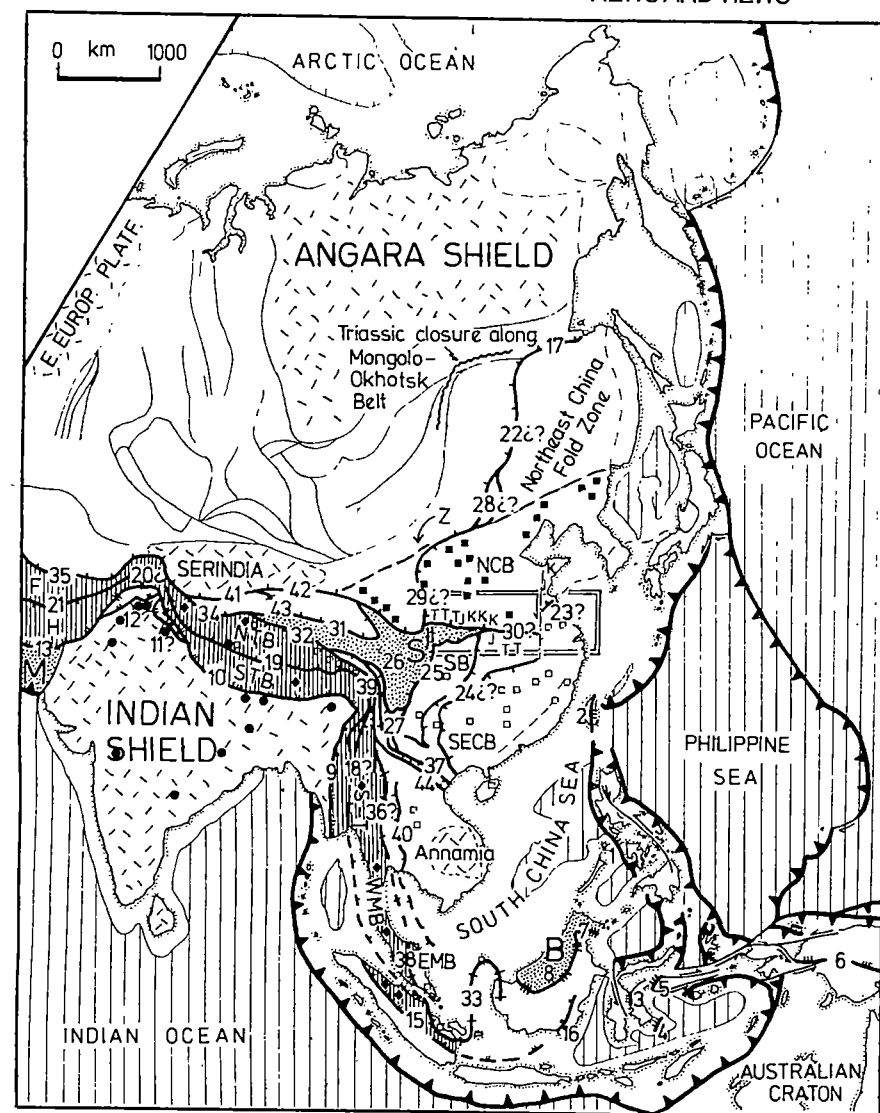
Mattauer *et al.*⁷ divide the Qin-Ling Shan into five longitudinal zones characterized by distinct rock assemblages and separated by major steep faults. In zones 1 through 4 (from north to south) they recognize a 'Caledonian' collision belt (an unfortunate feature of the paper by Mattauer *et al.* is the employment of obsolete and misleading Stellerian phase nomenclature that they seem to have inherited from their Chinese sources), and in the most southerly zone 5 they see an Indonesian intracontinental fold and thrust belt. They

also recognize a 'Hercynian' deformation and metamorphism in zones 2 through 4, that are also viewed as intracontinental, that is, post-collisional.

The implied view that the North and South China block had already collided by the Devonian represents a minority opinion even among Chinese geologists and conflicts with other evidence from palaeobiogeography¹⁹ (see figure) and palaeomagnetism^{20,21}. In my view, the geological map provided by Mattauer *et al.*⁷ does not contain cartographic evidence for a Devonian or even a late Palaeozoic collision along the Qin-Ling mountains (for instance, there is apparently no unconformable molasse of appropriate age nor any intrusion earlier than late Palaeozoic cutting the structures discordantly). Because the available evidence in the published literature overwhelmingly favours a Triassic collision^{4,13-20}, I obtained an independent opinion from K.J. Hsü, who had visited the same area as Mattauer *et al.* earlier this year under the guidance of Chinese geologists.

Hsü believes that the Qin-Ling Shan could be interpreted as a collision-type mountain belt, comparable to the Alps, that culminated in late Triassic time ('Indonesian'). He thinks that the stratigraphic evidence indicates only one orogenic cycle (Wilson cycle) stretching from the late Proterozoic to the late Triassic. Zone 1 of Mattauer *et al.* is viewed by Hsü as an Austroalpine-type rigid basement nappe, whose main internal deformation had already occurred probably in late Proterozoic time. In zone 2, Hsü sees Pennine-type folded nappes of basement material, whereas in the 'Central Qin-Ling Dome' (zone 3) an equivalent to the *schistes lustrés* and ophiolites is seen. Hsü, like Mattauer *et al.*, interprets this as the remnants of a former ocean. He believes the so-called 'Devonian trough' (zone 4) was a region of turbidite sedimentation, but disputes the Devonian age assigned to the turbidites here, for that age comes from macrofossils in exotic limestone blocks found in the turbidites. Hsü points out that Triassic ammonites have been recovered from similar rocks in the same zone farther west, thus making at least some parts of the turbidite sequence of the 'Devonian trough' Triassic.

The Triassic to early Cretaceous age of the main linear granite belt north of the Qin-Ling Shan, the 'Variscan' age of the earliest known subduction-related magmatism ('Variscan' or 'Hercynian' in Asia may in places reach well into the Triassic, emphasizing once more the misleading consequences of the employment of these terms outside their European type areas), and the pronounced difference both in the palaeophytogeographical and in the palaeomagnetic²⁰ signature of the Permo-Carboniferous rocks on both sides of the Qin-Ling Shan, all seem to converge to support a Triassic collision. This becomes



- Sutures of the Tethysides
 — Cimmeride (Palaeo-Tethyan) sutures
 — Alpine (Neo-Tethyan) sutures
 — Circum-Pacific sutures
 — 28? Location & age of suture disputed
 — 23? Age of suture disputed
 — 20? Location of suture disputed
 — Baykalian & Palaeozoic sutures outside the Tethysides
 — Mesozoic sutures outside the Tethysides
 — Active subduction (teeth on upper plate)
 — Active strike-slip faults
 — Oceanic flysch/mélange fill
 — Oceanic area
 — Glaciogenic sediment of Permo-Carboniferous age (outside Indian continent)
 — Fragments of Cimmerian Continent
 — Triassic } granites of Qin-Ling & Dabie Shan
 — Jurassic }
 — Cretaceous }
 — Cathaysia (North) } flora
 — Cathaysia (South) }
 — Gondwana }
- Main Precambrian continents

Tectonic map of Asia showing the Phanerozoic sutures; Tethyside sutures, of which the Qin-Ling Shan (boxed area) is a part, are emphasized. The map also shows the distribution of late Palaeozoic (Permo-Carboniferous) glaciogenic sediment and flora distribution (except the Angara flora) to identify independent blocks of that age and their possible palaeobiogeographic affiliations. Notice how sharply the Qin-Ling suture separates the North Cathaysia realm from the South Cathaysia realm. Also notice the pronounced Triassic to Cretaceous batholith belt immediately north of the Qin-Ling suture.

Numbers refer to sutures: (1) Mindoro; (2) Taiwan; (3) Poso; (4) Butung; (5) Peleng; (6) New Guinea/Papua; (7) Sabah; (8) Sibiu; (9) Burma; (10) Indus-Yarlung Zangbo; (11) Shiok; (12) South Ladakh; (13) Waziristan; (14) Makran; (15) Sumatran; (16) Meratus; (17) Shiika; (18) Sittang Valley-Myitkyina; (19) Banggong Co-Nu Jiang; (20) Rushan-Pshart; (21) Waşer (Farah-Rud); (22) Da Hinggan Ling; (23) Huang Shan; (24) Central South China; (25) Longmen Shan; (26) Litang; (27) Jinsha Jiang; (28) Inner Mongolian; (29) West Ordos; (30) Qin-Ling; (31) Anyemaqen Shan; (32) Hoh Xil Shan; (33) Natuna; (34) Suture of the southern synclinorium of the western Kuen-Lun; (35) Herat; (36) West Thailand (Nan-Uttaradit); (37) Song Da; (38) Medial Malaya (Bentong-Raub); (39) Lancan Jiang; (40) Petchabun; (41) Suture of the northern synclinorium of the western Kuen-Lun; (42) Qilian Shan; (43) Qimantag; (44) Song Ma. Lettering: (B) Borneo accretionary complex; (EMB) East Malaya Block; (M) Makran accretionary complex; (NCB) North China Block; (NTB) North Tibetan Block; (S) Songpan-Ganzi accretionary complex; (SB) Sichuan Block; (SECB) Southeast China Block (SB+SECB=South China Block); (Z) Li Chunyu *et al.*'s North China suture (ref. 14). The map is compiled from data in refs 4 and 19.

the more plausible when one follows the suture both westwards into the Kuen Lun and eastwards into Korea, where it continues into the orogens of Triassic age^{4,14,16}.

A very significant point made by Mattauer *et al.*⁷ and Peltzer *et al.*⁸ is the presence of significant strike-slip motion along the Qin-Ling Shan in both Tertiary-Quaternary time and probably also earlier. The post-Eocene movement, of the order of several hundred kilometres, is rightly ascribed to the effects of the Himalayan collision. But the possibility of a few thousands of kilometres of motion during the Mesozoic (see ref. 20) presents acute problems in attempting palaeogeographic and palaeotectonic reconstructions. Thus, the isotopic or palaeontological ages obtained from slivers, or even entire terranes, dragged along such giant strike-slip systems may have no relevance to the orogenic history of the edifice in which they now lie embedded.

The two papers by Mattauer *et al.* and Peltzer *et al.* clearly indicate, in the light of the above discussion, that the Qin-Ling Shan has had an extremely complicated, multi-stage evolution, during which its constituent elements may have been repeatedly redistributed by several episodes of important strike-slip motion along the belt. This alone may help us to see why the interpretation of the tectonic history of this orogen is so difficult and prone to controversy. This must be true, albeit in different degrees and in different combinations, along all of the suture zones that hold together the Asian edifice, showing²² how dangerous and misleading it must be to consider orogenic evolution along cross sections alone. Much more work remains to be done before even a glimpse is obtained of the problems that are involved in understanding the orogenic evolution of the Qin-Ling Shan, as well as the assembly and disruption of Asia. □

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Cosmology

Radio supernovae as probes for Hubble's constant

from Alan P. Marscher

IT SEEMS remarkable that, over half a century after Edwin Hubble discovered that all distant galaxies are receding from us with velocities proportional to their distances, astronomers still cannot agree on the value of Hubble's constant, the slope of this relationship. This important parameter corresponds to the expansion rate of the Universe in most cosmological models, and can be used to determine the present age of the Universe in the standard big bang model. On page 25 of this issue, a new method, which utilizes the observed expansion rate of radio-bright supernovae to estimate Hubble's constant, is reported by Bartel and his colleagues¹. The method, while somewhat dependent on a model, obviates the systematic uncertainties inherent in optical distance determinations.

Using optical distance indicators, Sandage and Tammann² arrived at a value $H_0 \approx 50 \text{ km s}^{-1} \text{ Mpc}^{-1}$ for Hubble's constant, with an estimated upper limit of about 60. On the other hand, de Vaucouleurs³ and Bothun and colleagues⁴ obtained values between 90 and 100, with a lower limit of about 85. That these two limits are so incompatible illustrates the importance of systematic errors in the derivation of H_0 (see ref. 2 for an excellent summary). Nearly all critical distance determinations depend on the use of very few distance indicators (such as Cepheid variables) to obtain the distances to more remote galaxies. Therefore, uncertainties in the use of this small number of primary distance indicators become systematic errors in the determination of H_0 . What is clearly needed is a method independent of the derived distances of local group galaxies.

Fritz Zwicky maintained that supernovae were the only worthwhile extragalactic distance indicators. Whether or not this proves to be the case, supernovae

at least have the advantage that their luminosities are high enough to allow detection at distances greater than 1,000 Mpc. It is also well established that they represent exploding stars with expansion velocities that can be measured via the Doppler shifts of the emission and absorption lines in the optical spectrum. One method for calculating the distance to a supernova, attributed to W. Baade and A. Weselink, uses the observed expansion velocity and the assumption that the optical-infrared continuum represents black-body emission from an opaque, expanding sphere of gas. The shape of the continuum yields the temperature of the black body which, together with the radius calculated by multiplying the expansion velocity by the time since the onset of the explosion, allows one to calculate the luminosity. The relationship between this derived luminosity and the observed flux depends only on distance, which can then be determined. The main difficulty with this method is that the optical-infrared continuum does not fit a black-body curve precisely and the deviations must be modelled theoretically for the method to be applied successfully⁵.

Bartel *et al.*¹ use what at first glance appears to be a much more straightforward approach: by using centimetric-wavelength, very-long baseline interferometry (VLBI), they have been able to measure the angular diameter of the radio source associated with supernova 1979c in the galaxy M100 in the Virgo cluster. Since the true diameter of the supernova can be derived from the optically-determined expansion velocity, the distance to the supernova (and the galaxy containing it) can be readily calculated. Using this distance to M100, they derive a value of $H_0 = 65^{+35}_{-25} \text{ km s}^{-1} \text{ Mpc}^{-1}$. While this range of possible values hardly solves

the controversy over Hubble's constant, it is exciting that an attempt has been made to employ such an apparently straightforward technique.

The large uncertainty in the value of H_0 obtained by this method arises mainly from two distinct difficulties. The first is observational. Extragalactic supernovae are faint at radio wavelengths, so much so that only a handful have been detected by the most sensitive radio instruments available. The successful use of VLBI requires a strong detection by at least 3 or 4 antennas, but with the current VLBI configuration supernova 1979c is only weakly detected. The combination of the very-long baseline array (to be built in the United States by about 1990) and large European radio dishes should improve this situation substantially.

The theoretical difficulty is a little stickier. The optical expansion velocity is only relevant here if the radio emission is co-spatial with the optical spectral-line emission. In fact, the leading model⁶ for the radio light curves of supernovae places the radio emission in a shock wave outside the bulk of the debris from the explosion. Bartel *et al.*¹ assume that the radio brightness distribution lies somewhere between that of a uniform sphere and that of a ring with an expansion velocity closely related to that obtained from the optical spectra. It has been shown⁷ that these extreme cases lead to values of H_0 which bracket those obtained using more realistic brightness distributions. This uncertainty in the appropriate geometry leads to the wide range of values for H_0 allowed by the data of Bartel *et al.* Eventually, the actual radio-brightness distribution of a supernova might be measured directly using VLBI, obviating the need to rely on theory. Even then, frequency-dependent apparent diameters (possibly observed by Bartel *et al.*), and other complications, might limit the ultimate accuracy of the method.

If, as expected, further refinements and continued observation improve the accuracy of the new technique, we will be blessed with a cosmological probe that is independent of all the systematic uncertainties that haunt the current optical methods. Such a breakthrough would enable cosmologists to get on with their business of model making, released from the nagging fear that the size scale and age of the Universe they have adopted could be off by a factor of two. □

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100 years ago

HYDROPHOBIA

ONCE more M. Pasteur is attracting the attention of the civilised world by his brilliant investigations . . . a boy of nine years old had been terribly worried on the 4th of last July; not only bitten in parts covered by his clothes but also on the hands. He was rescued covered with foam, and bleeding from no less than fourteen wounds. There was no question that the dog was mad, and in all human probability this child, Joseph Meister, was doomed to a certain and horrible death. M. Pasteur felt himself justified in applying the means to this suffering fellow creature, which had already proved efficacious in the case of brutes.

The inoculations were made with a subcutaneous needle, began on the 7th, and were

concluded on the 16th of July. "Control experiments" were made with the same injections upon rabbits, and proved that the virus was active. Moreover, since the effects of the modified virus, when introduced into an unprotected animal, are rapid and severe, and its period of incubation extremely short, the result of the attempt to rescue the child from a horrible death would soon be apparent. If he had died of hydrophobia, it would probably have been within a month. If he survived this period there was every reason to hope that he would be as much protected against its future manifestation as the dogs which had been tested before.

Joseph Meister was in perfect health at the end of August, at the end of September, and at the end of October. M. Pasteur believes that he is safe from hydrophobia for the rest of his life. From *Nature* **33** 1, 5 November 1885.

A new protein sequence data bank

SIR—A protein sequence data bank, called PGtrans, is available from our laboratory. This data bank is generated by automatic computer translation of the well-known nucleotide sequence library GenBank (established by Bolt, Beranek and Newman Inc. and Los Alamos National Laboratory). For this we designed a translator program which scans and interprets the specific information fields of each GenBank entry: DEFINITION, SOURCE and FEATURE table. The translator program can thus decide if the corresponding nucleotide sequence includes a coding region, and what is the proper genetic code to use to translate it into a putative protein sequence.

For each coding region successfully translated, the program creates a PGtrans entry including a text constituted of several fields: CODE, NAME, SOURCE, REFERENCE and COMMENT, followed by the amino-acid sequence. The translator program checks for unexpected 'stop' codons eventually found in coding regions. If a 'frame shift' allows the errored coding region to be rescued, the tentative amino-acid sequence is included in PGtrans. However, a warning is included into the COMMENT field to indicate a potential problem. The current PGtrans databank corresponding to GenBank (35.0) contains 3,107 entries for a total of 483,649 residues. Figure 1 shows the

entry-length distribution for PGtrans 29.0. A listing of the encounter anomalies is routinely communicated to the makers of GenBank.

The main purpose of PGtrans is to offer a direct access to all amino-acid sequences coded among GenBank nucleotide sequences and thus to be an efficient tool for protein homology searches. Its format is compatible with the rest of our sequence analysis software¹ and will be consistent with the recommendations of the CODATA task group on protein sequence data banks². Since we conserved the minimum of text information necessary to identify the protein, PGtrans is a compact data bank (1.4 Mbytes) which could fit for few more years into microcomputer environments. Among other efforts in gathering protein sequence data³⁻⁵, the role of PGtrans is to provide a rapid update of new protein sequences determined by nucleic acid analysis. Magnetic tape copies (card image) of PGtrans will be distributed free of charge upon request to our laboratory.

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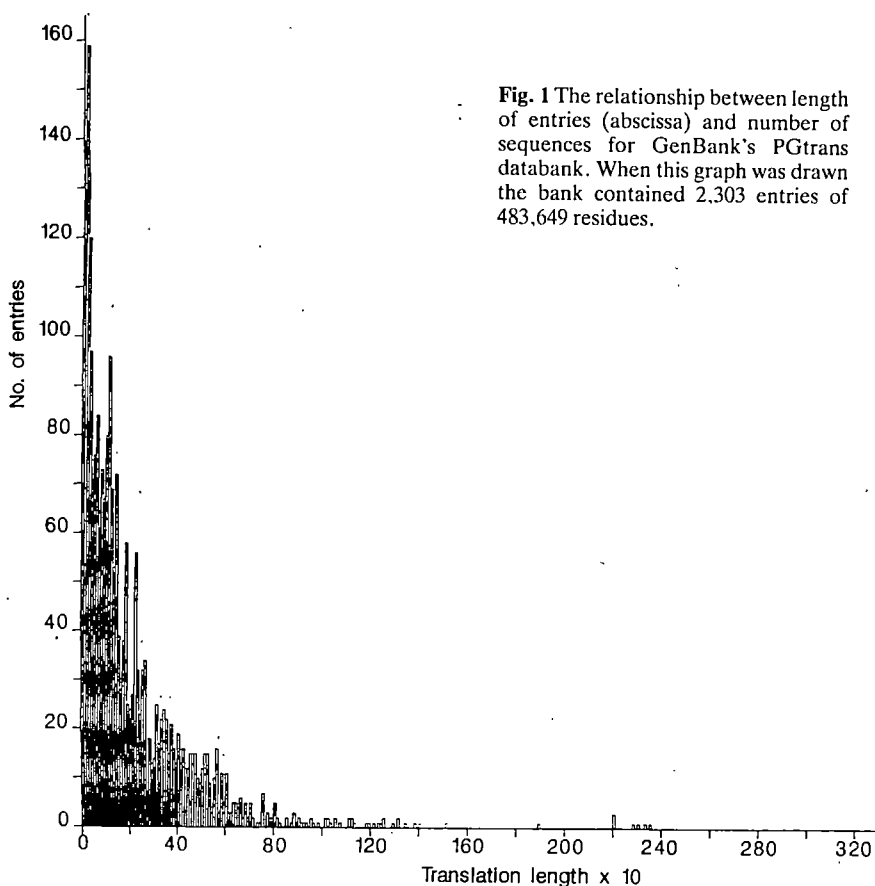


Fig. 1 The relationship between length of entries (abscissa) and number of sequences for GenBank's PGtrans databank. When this graph was drawn the bank contained 2,303 entries of 483,649 residues.

Three-dimensional electron lattice

SIR—*Nature* reported on 14 February (313, 527; 1985) the discovery of a three-dimensional electron lattice in *n*-HgCdTe by Rosenbaum *et al.* (*Phys. Rev. Lett.* **54**, 241; 1985). In fact the discovery was made in my laboratory in 1979. Our response to Rosenbaum *et al.* and relevant references can be found in *Physical Review Letters* for 22 July (55, 443; 1985).

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Shadow boxing with Darwin

SIR—Dawkins's taunting review¹ of Eldredge's book *Time Frames: The rethinking of Darwinian evolution and the theory of punctuated equilibria* raised important issues of evolutionary theory that can only but invite comment.

There always have been two components to Eldredge and Gould's theory of punctuated equilibria, as the phrase suggests explicitly — 'long periods of stasis (equilibria) punctuated by relatively much shorter periods of change. To reiterate, yet again, that the periods of change potentially fall within traditional boundaries of gradual Darwinian evolution by means of natural selection, and to ignore the major periods of stasis (a device which allows Dawkins to dismiss the whole concept as an "interesting little theory . . . lying firmly within the neo-Darwinian synthesis") reduces scientific evaluation to a parlour game of one's own invention, which whilst entertaining and superficially coherent, does not offer proper scientific guidance for the general reader.

If we accept, for the moment, that stasis is a widespread observation in the geological record, and if too we accept that the forces behind selection are the only means for promoting biologically useful novelties throughout a population (setting aside contemporary knowledge that the molecular behaviour of the genes can effect similar changes), then it is surprising that little significant evolution occurs over periods of time that may span from hundreds of thousands to millions of generations, in a diverse range of species. The point is not that evolution is gradual "during the times that it is actually happening" (Dawkins' emphasis) but that it seemingly is not happening for most of the time. This was the message of the infamous Chicago meeting, which many of us took home, and which demands an explanation.

Attempts to bring stasis within the neo-Darwinian synthesis (see ref. 2 for the best of them) are not wholly satisfactory in that they plead for a general process called stabilizing selection, coupled to an

assumed inertia of developmental systems, applicable to all examples of stasis irrespective of species or conditions. This says that no evolutionarily significant shift in the average phenotype of a population took place because the ecological and developmental conditions were such that mutationally deviant individuals were continually selected against. This explanation, whilst theoretically self-evident, presupposes a uniformity of environment over vast distances of time and space that surely must be unacceptable to ecologists familiar with the ever-changing web of relationships that go into the making of ecological niches. It is difficult to accept that the heterogeneity of niches, once set up, is so stable and no longer subject to further subdivision that the usual ecological pressures, forcing variant individuals to selectively adapt to progressively specialized niches, no longer exist. Furthermore, developmental inertia is becoming the panacea for all our unsolved problems. When we don't want any appreciative directional selection to be taking place we appeal to the severity of genetic and epigenetic constraints implicated in the development of a single-celled zygote to a multicellular adult. When we do need to explain, nevertheless, that evolution is seen to be taking place sometimes, such constraints miraculously disappear.

There are other potential explanations, Darwinian and non-Darwinian, for stasis and its occasional interruption, which have not been fully explored, as yet^{3,4}. However, notwithstanding all such attempts, our general ignorance of developmental genetics and our even greater ignorance of the ecological flux and microheterogeneity of past environments, simply make it premature to wage polemical wars and to demote contemporary observations and their interpretation as "a technical parochial affair if ever there was one". The writings of Darwin, and indeed those that contributed to the formulation of the neo-Darwinian synthesis in the 1930s, are not Old Testament tracts to be poured over by armchair exegesis. They cannot supply, without resorting to too many *ad hoc* assumptions, all the answers to the multiple causes of the ebb and flow of evolution. This is relevant not only to the tempo of change observed in the geological record, but also to the evolutionary consequences of the unexpected molecular dynamics of the genes themselves. Physics moved on from Newton, and biology might need to move on from Darwin, if we are to explain, satisfactorily, all that we observe.

Dawkins is defending a tradition which hardly needs defending (for contemporary theories whilst non-Darwinian are not anti-Darwinian, despite the journalistic, and occasional professional, recourse to hype), when at the same time he is getting a little of his own back on the justifiable attacks on his own interesting little misjudgement of the "gene as the unit of

selection" (selfish gene). Ironically, this technical blunder, (despite Dawkins' protestation that it "has enriched the synthetic theory"), stood Darwin on his head and sent many a true Darwinian rushing to set the old gent firmly on his feet. Dawkins' style seems to be a self-conscious attempt to emulate the rapier-like thrusts of Sir Peter Medawar in his prime, who demolished bogus scientists with unremitting glee. Unlike Sir Peter, however, we are left with the image of a man who, in largely ignoring the real scientific issue under review, is shadow boxing with himself: a stance not much help for an informed evaluation of evolutionary processes.

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The length of myosin subfragment-one

SIR—A recent *News and Views* comment¹ on electron microscopy² of crystals of isolated myosin heads (S1) emphasized that these results yielded a length supporting earlier rotary shadowed views of intact myosin and S1 molecules. Results obtained from X-ray scattering³ and (by implication) certain electron microscopic acto-S1 reconstructions which yielded shorter structures were rejected as incorrect. We contend that because of a confusion arising from comparing two different kinds of lengths, there is little or no evidence for either support or rejection.

Winkelmann *et al.*² report at least 160 Å for the 'length' of S1. Measurements from their projected images (corrected for depth) indicate that this is a *contour length* and that the *maximum chord* (l_{\max}) observable within their structure is 120–140 Å long. The lower limit arises from the contour drawn in Fig. 11a of ref. 2. The upper limit derives from the assumption that there are crystal contacts along the long direction of the molecule. While we agree that the contour in Fig. 11a probably underestimates the volume, the data do not necessarily lead to the conclusion that the missing mass is along the long direction of S1; the molecule could just as well pack in the crystal without contacts along this direction.

X-ray scattering results give an l_{\max} = 120 ± 10 Å and acto-S1 reconstructions give l_{\max} = 115–150 Å (see Table 1 of ref. 4) which are consistent with the crystal values. (The largest value in this range arises from scallop S1 with regulatory light chain.) Although these results, as well as the crystal results, could underestimate the true length of the myosin head (for different reasons), the current S1 crystal results do not require that the l_{\max} (190 Å) of the entire myosin head^{5,6} or S1⁷ esti-

mated from rotary shadowing projections be correct. It is nevertheless encouraging that there are features of S1 on which nearly all results concur: a curved shape with most of the mass located near one end.

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Control of the cell cycle in yeast

SIR—In a recent *News and Views* article on yeast cell biology (*Nature* **316**, 678), I. Herskowitz states that mutations in yeast that result in G_1 arrest with reduced protein synthesis are relatively unlikely to reflect specific cell-cycle controls, in contrast with mutations such as *cdc28*, which induce G_1 arrest without reduced protein synthesis. There are several reasons for viewing this statement with caution.

First, successfully meeting the criteria of continued protein synthesis for G_1 arrest mutants does not exclude the possibility that the mutation may effect some continuous aspect of metabolism unrelated to growth control *per se*. For example, a mutation in the gene encoding ribonucleotide reductase could in principle mimic the effect of exogenously applied hydroxyurea, which can lead to G_1 arrest with continued protein synthesis.

Second, there is a wide body of evidence which suggests that attainment of a critical amount of protein is necessary for traversal of the G_1 period, and it seems relatively safe to assume that cell cycle control points operate both before and after such an event. There is no *a priori* reason that physiologically important cell cycle controls are more likely to operate after this event; in mammalian cells, G_1 arrest induced by depletion of polypeptide growth factors is usually accompanied by a marked fall in protein synthesis.

Finally, two of the mutations in *S. cerevisiae* that lead to G_1 arrest with reduction of protein synthesis, *cdc25* and *cdc35*, are now thought to be specific signal transduction components of the cAMP pathway. These gene products might exert their growth-regulatory effect by influencing protein synthesis, or a feedback mechanism might slow protein synthesis in response to inactivation of these gene products. In any case, any comprehensive view of cell cycle control should not exclude such regulatory proteins from consideration.

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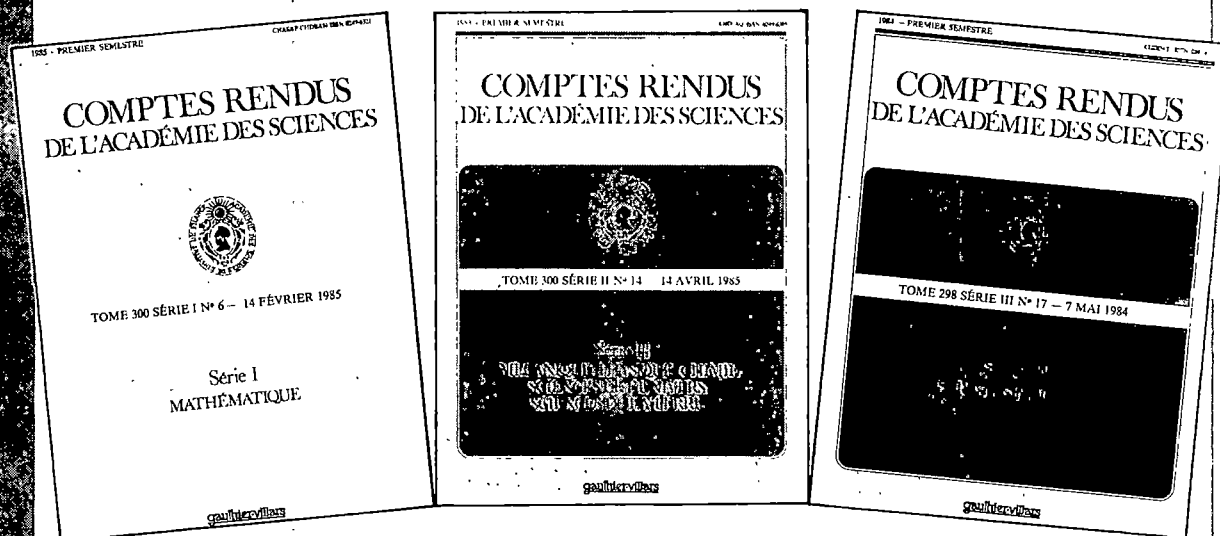
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The politics of pesticides

Eric Ashby

Pesticides and Nature Conservation: The British Experience 1950 – 1975.

By John Sheail.

Oxford University Press: 1985. Pp.276. £19.50, \$29.95.

THE various messages conveyed by this book are important. But just as important are the seven pages at the end which carry the sources from which it was written — nearly 300 references, practically all of them from the files of the Nature Conservancy, a body set up under royal charter as the fourth of Britain's research councils in 1949 (added to the Department of Scientific and Industrial Research, the Medical Research Council and the Agricultural Research Council).

What is unusual about that? It is that the files were opened to John Sheail, even though most of them were less than 30 years old (in Britain, a ban prevents access to the papers of official government organizations for that period). How this miracle of diplomacy was achieved in a country that has no Freedom of Information Act, and which is paranoid about the disclosure of information less than 30 years old, the author does not explain. But the benefits of this relaxation are clear. The recent history of pesticide control is now public knowledge; in part, at any rate, for of course the files of the relevant Ministries may remain closed until the year 2015 (for matters being discussed today) and the files of the industries that produced pesticides are never likely to see the light.

The value of having this sort of information made public is that people concerned to influence environmental policy can find guidance on how to do it and (from many episodes described in this book) how *not* to do it. Nothing but good can come from disclosure of the complexities and legitimate conflicts which have to be resolved before decisions about the environment can be made (though only a reckless optimist would hope that the Department of the Environment might be shamed into releasing some of its papers to historians). In this sense the book is a landmark and those who had the courage to authorize the files to be opened are to be congratulated.

Skimmed over, the book itself is a sober, detailed account of the use and control of insecticides and herbicides in Britain, as seen by the Nature Conservancy and with emphasis upon the part played by the Conservancy in solving the scientific problems and interpreting the solutions for politicians. In rough outline it is a familiar story, for "every schoolboy knows" the gist of the message in Rachel Carson's *Silent Spring*. But to put the book aside with the conclusion that it is no more than a parochial account of Britain's

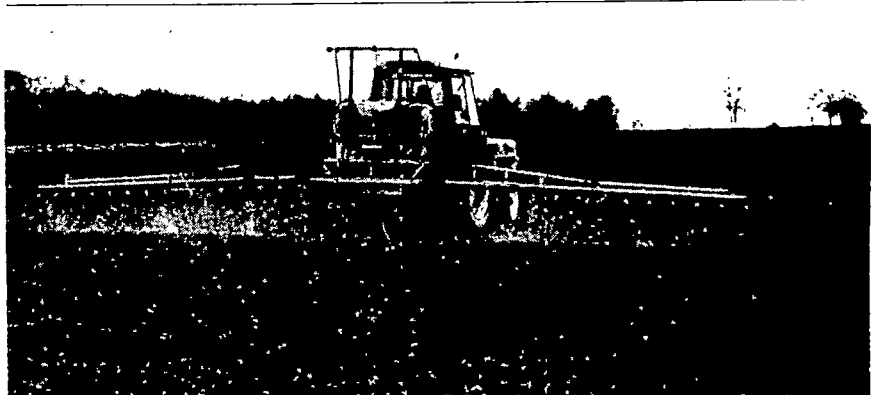
experience in pesticide control would be a misjudgement. True, it is a case-study of environmental decisions for one narrow issue in one small country. But it is out of such case-studies that general principles emerge, and the intricate details demand close reading: the distinction between correlation and causation in field observations; the subordinate part scientific evidence plays in some political decisions; the resolution of conflicting self-interests when there is "right" on both sides.

The story Sheail has to tell is best illustrated by the several themes which characterize his case-study. One of them is the difficulty of linking cause and effect in ecology. Forty years ago the *Atlantic Monthly* published an article by V.B. Wigglesworth, a British entomologist, about the possible side effects of DDT; it might, thought Wigglesworth, kill the predators of the very pests we want to destroy, when the predators might, in the long term, be more effective agents of control. It was only concern that DDT might be a hazard to human health — not to other organisms — that prompted official enquiry into its effects, and not until the 1950s that Wigglesworth's warning took root. By that time, however, it was clear that millions of pounds worth of crops were being saved in England and Wales by the application of insecticides, and large profits were being made by the manufacturers of sprays and equipment. There would have to be compelling reasons for any government to restrict this practice.

It was left to the Conservancy and to voluntary bodies such as the Royal Society for the Protection of Birds to find those reasons. There was an increase in the numbers of dead birds and mammals found after spraying; but it was easy to explain this away by saying that the public-

ity given to bird-deaths stimulated volunteers to look for dead birds, and the cause of the increase in numbers of dead birds was simply the increase in the numbers of people looking for them. Challenged to produce credible evidence that the birds and mammals had been killed by pesticides the Conservancy was obliged to set up its own research unit, with pitifully inadequate funds, to tackle problems which were insoluble without more refined techniques in analytical chemistry than were available at the time. It was not until the early 1960s that the Conservancy's research unit was able to publish analyses for pesticide residues in the bodies of dead birds, showing that these residues were higher among raptorial and fish-feeding birds, whose diet was contaminated by lower concentrations of the residues; it confirmed the hypothesis of concentration of residues in the food-cycle. The peregrine falcon was the protagonist in this drama. Its principal prey was live birds; these (with the irony of Greek drama) were the falcon's undoing. By 1962 its pre-war population was halved. But by then the government had accepted the scientific evidence. Persistent organochlorine pesticides were restricted, and the peregrine falcon population is well on the way to recovery.

A second theme of the book is the contribution voluntary bodies have made to the control of pesticides. It was a joint committee of the British Trust for Ornithology and the Royal Society for the Protection of Birds that hired chemists to do post-mortems on birds thought to be killed by organochlorines or mercury on seed dressings. The committee was able to show a clear correlation between sowing programmes and mortality in many parts of England. The record of voluntary conservation bodies in Britain is in every way as impressive as the efforts of the Sierra Club and the Audubon Society in America, but with this difference: in Britain the voluntary bodies have worked by bringing informal pressure to bear on the government and the public, while in the United States the voluntary bodies have frequently had to resort to law. In the United States the battle against organochlorine



Crop spraying with pesticide — the story is not over yet.

pesticides, for example, was won through the Environmental Defense Fund.

A third theme also is concerned with voluntary effort, and it is peculiarly British. The control of pesticides comes under a so-called Pesticides Safety Precautions Scheme which has persuaded the manufacturers voluntarily to abandon some pesticides altogether. From time to time governments are pressed to impose mandatory control (and there is still a need for it in some non-agricultural uses of pesticides), but every time the matter is looked into, the advice given (even by the Royal Commission on Environmental Pollution in its Fourth Report in 1974) is that there is no case for replacing the voluntary system.

Finally, Sheail's book has some vivid illustrations of the familiar pattern of events in environmental politics, a pattern which anyone who wants to take part in such activities has to understand. There is a prologue of silent scientific work to which politicians, even if they know about it, are indifferent. There follows a sensa-

tional episode, such as the *Torrey Canyon* disaster, or an emotive popularization of some hazard, such as was so successfully done in *Silent Spring*. (It was the chairman of a Congressional subcommittee on agriculture who described the public opinion left by *Silent Spring* as "the worst residue problem we have to face today".) The cautious scientists, striving to solve the problems, are often bitterly criticized during this emotive phase. Then come pressures for legislation or, in the United States, lawsuits; and finally there has to be a compromise between those who want to exploit the environment for profit (either by growing crops or making pesticides) and those who want to protect it.

Above all, John Sheail's book is a record of missionary work by scientists among civil servants and politicians. There is no climax, but nor should there be: the story is not over yet. □

Lord Ashby is a Fellow of Clare College, Cambridge CB2 1TL, UK. He was Chairman of the Royal Commission on Environmental Pollution from 1970 to 1973.

Viewed from above

Melbourne G. Briscoe

Introduction to Satellite Oceanography. By G.A. Maul. *Martinus Nijhoff*: 1985. Pp.606. Dfl.220, \$80.

Satellite Oceanography: An Introduction for Oceanographers and Remote-sensing Scientists. By I.S. Robinson. *Ellis Horwood*: 1985. Pp.455. £42.50, \$63.75.

Methods of Satellite Oceanography. By Robert H. Stewart. *University of California Press*: 1985. Pp.368. \$38.50, £32.75.

IT WAS not so long ago that the proper background for a physical oceanographer was deemed to lie more in one's heritage and sea legs than in any particular formal education. Experience and practical apprenticeship were far more important than classes and books. In recent years it has been seen to be necessary to complement that approach of thoughtful explanation and observation with a good dose of common sense about electronic and mechanical instruments, and a solid grounding in mathematics and physics. Such mandatory education now includes the need for more than a passing knowledge of numerical methods, thermodynamics, synoptic and boundary-layer meteorology, and, depending on the chosen specialization, chemistry, geology and biology.

In this rapid evolution and expansion of formal training, however, it has been easy to forget that oceanography — even today's physical oceanography of potential vorticity balances and dispersion relations — is still mainly an observational science. Our ideas about how the ocean works are driven by what we see of its workings.

These three books bring this issue back into the limelight. They all attempt to display and explain the enormous potential of remote sensing of the oceans, principally from satellites, and to provide the information one might need to use and to understand this newly available tool. Maul aims his book at "a wide range of oceanographers, managers, and engineers" (is this a hierarchy, I wonder, and, if so, which way does it go?); Stewart says his is for "scientists with varied backgrounds"; and Robinson addresses his more eclectic book to an equally broad audience: "practising oceanographers . . . sensor technologists and remote-sensing specialists . . . and students of both oceanography and remote sensing".

The content and success of each of the books reflect the authors' backgrounds. Maul and Robinson both specialize in the infrared and optical properties (such as colour) of the sea surface, while Stewart concentrates on radio and radar scattering from the surface. Robinson's book provides some useful material on orbits and data handling, much of which is probably ephemeral, whereas Stewart has tried to include fundamental and perhaps more durable material about the basic physics of the sensors and the associated electromagnetic emission and scattering processes. Stewart's book is the most pleasing typographically, and includes a number of impressive colour plates which illustrate certain applications. Maul contains considerable detail and comes the closest to being self-contained on a number of topics. Robinson gives a lot of help to the person actually working with the data (not one of his intended audience, by the way), but a large number of typographical errors lessens one's confidence in some of his material.

In books such as these, it is unlikely that the reader will start at the beginning and read to the end; a comprehensive and detailed index is therefore essential. As a test, I tried to look up the established accuracy of sea surface temperatures as measured from a satellite, which is one of the oldest and most useful products of remote sensing. Only Robinson allows direct access to the topic via the index (but under the heading "SST"). Stewart does not even have an entry under "Temperature", although his discussion of the topic is arguably the best of the three. (Apparently, if one works very hard, it is possible to get about 1K accuracy.)

The authors are all successful in their goal of making remote sensing more accessible to the oceanographer, perhaps more so as textbooks — they are all based on course notes — than as reference books. Robinson, however, mostly fails in his attempt to provide an oceanographic background for the remote sensor; an entire book would be necessary for this task, and would be very difficult to write, but such a volume is clearly needed to stem the trend of space engineers publishing second-rate oceanography in non-oceanographic journals and in non-refereed conference proceedings.

But I wonder if these books are really necessary. Even if they meet their stated goals, was the goal worth addressing? Traditionally, a new tool becomes known to the science community by its being used and by publication of the results in science journals. These authors argue, however, that here the tool (sensors on satellites) is so different, so complicated and so inaccessible in terms of its construction and its data handling, that the issue must be forced, rather than just being allowed to happen. I suppose they are right, but you cannot force good science and it is good science based on remote sensing that will ultimately sell the new tool.

There is considerable promise in satellite oceanography as a broad but not detailed observational tool; this is precisely the missing dimension in traditional oceanographic measurements, so with perseverance by all and tolerance by the traditionalists this new view may yet pay off. These books are a step in the right direction. □

Melbourne G. Briscoe is an Associate Scientist in the Physical Oceanography Department, Woods Hole Oceanographic Institution, Woods Hole, Massachusetts 02543, USA.

New in paperback

• *The Nature of Selection: Evolutionary Theory in Philosophical Focus* by Elliott Sober. Publisher is MIT Press, price \$9.95, £9.95. For review see *Nature* 314, 680 (1985).

• *Climate and History: Studies in Past Climates and Their Impact on Man* edited by T. M. L. Wigley, M. J. Ingram and G. Farmer. Publisher is Cambridge University Press, price £15, \$24.95. For review see *Nature* 298, 499 (1982).

Picking the winners by number

David E.H. Jones

The Awards of Science and Other Essays. By Eugene Garfield. ISI Press, Philadelphia/STM Distribution, Enterprise House, Ashford Road, Ashford, Middlesex TW15 1XB, UK: 1985. Pp.572. \$30, £26.

CURRENT CONTENTS, which every week reproduces the title pages of a selection of learned journals, must be the tersest and most functional magazine in existence. Almost its only concession to literature (as opposed to *the literature*) is the section "Current Comments", in which the editor, Eugene Garfield, looks up from his photocopier and discourses on whatever topic has taken his fancy that week. He has been publishing collections of his "Comments" since 1973, under the general title *Essays of an Information Scientist*; this book is the seventh in the series.

Of the 61 essays here reprinted, nine deal with recent research on biomedical topics such as cystitis and gerontology, and ten are tributes to notable information scientists or recent Nobel Prize winners. There is also a description of an artwork (with colour plate), a discussion of science books for children, and two articles by other authors which Garfield comments upon. But most of the essays deal with aspects of information science: in particular the services provided by the Institute for Scientific Information, founded by Garfield in 1960, and analyses of its compilations of scientific citations which are published as *The Science Citation Index*.

Most researchers, I imagine, use the *Index* regularly, if only to look themselves up and find out how they are doing: how often their most recent papers have been cited, and by whom. Computer technology not only enables the enormous lists of citations to be compiled and indexed, but also allows Garfield to indulge in his favourite hobby of scanning the lists for highly cited papers. Thus the book includes accounts of the hundred most cited papers ever; a hundred "citation-classics" from *The Lancet*, and another hundred from *The New England Journal of Medicine*; several compilations of the articles most cited between 1961 and 1982; and several essays on the citation-patterns of different scientific disciplines.

Each such essay contains a wealth of statistical material on papers and their citation-rates, the ranking of journals by citation-frequency in the rest of the literature, the number of citings of "classic" (highly-cited) papers as a function of time and so on. Garfield uses this material as a springboard for discussing developments in the field involved, and its current "hot

topics", usually buttressed by explanations from the principal authors concerned. Intriguing "maps" of the scientific frontiers are sometimes included, based on the flow of citations between the various authorities.

All this is splendid stuff, and gives a lot of information about the most active (or at least the most verbal) areas of science. However, Garfield's preoccupation with picking winners tends to give a one-sided impression of what science is all about. Thus the title essay "The Awards of Science" deals with just that; the scientific prizes and awards available to scientists, and how many of the recent prize-winners have been spotted among ISI's "most-cited authors". The object of the essay is to dispel the impression that the Nobel Prize is the only possible goal for a scientist — there are many other prestigious

prizes to compete for. "To set a practical limit for the essay, we have only included those awards that bestow an honorarium of at least \$15,000."

This image of science as a sort of competitive hit-parade is probably an occupational bias of the computerized citation-analyst. Fortunately Garfield's interest in science transcends this limited view. His concise accounts of research in many fields, and of the achievements of many scientists, are well worth browsing through for their erudition and informativeness — and, as one would expect, they are impeccably, not to say obsessively, documented and referenced.

David E.H. Jones is a guest staff member in the Department of Physical Chemistry, University of Newcastle upon Tyne, Newcastle upon Tyne NE1 7RU, and a science consultant to industry and the media.

The moving spirit

Simon D.M. White

Gravitational Physics of Stellar and Galactic Systems. By William C. Saslaw. Cambridge University Press: 1985. Pp.491. £50, \$90.

As William C. Saslaw points out in his new book, gravitation is the spirit which underlies almost all astronomical phenomena. Rightly, then, the study of gravitational dynamics has had a major part in astronomy since the time of Newton, and today it is the foundation on which we build our understanding of the structure, formation and evolution of planetary systems, of star clusters, of galaxies, and of the large-scale distribution of galaxies. Recent research has been greatly stimulated by the advent of computers that are able to integrate the equations of motion directly for systems containing thousands of particles. As a result gravitational dynamics has become, in some part at least, an experimental science.

In view of its importance in modern astronomy, it is surprising that there is no recent all-embracing account of the subject. *Gravitational Physics of Stellar and Galactic Systems* thus addresses itself to a well-recognized gap in the literature. As the title suggests, Saslaw is here more interested in elucidating physical processes than in modelling astronomical systems. His book is written in an easy-going conversational style, and is laid out as a survey of the phenomena which govern the gravitational evolution of many-body systems.

The whole field is divided into four general areas: homogeneous systems, infinite inhomogeneous systems, finite spherical systems and finite flattened systems. The first part discusses descriptive formalisms such as the Liouville, Boltzmann, Fokker-Planck and BBGKY hierarchy

equations, together with fundamental processes such as collisional relaxation, dynamical friction, collective scattering and Landau damping. The second part concentrates on clustering in an infinite universe, with particular emphasis on thermodynamic considerations, while the third deals with the structure of spherical systems and their evolution under the influence of collisional relaxation and binary formation. The last part discusses some aspects of disk galaxy dynamics and a few other assorted topics. The sophistication of treatment and completeness of coverage decrease steadily from one part to the next, even though the relevance of the subject matter to contemporary research increases. The book restricts itself to processes involving purely Newtonian gravity.

On the dust jacket, the book is described as suitable as a text for graduate students. However the depth and completeness of coverage are too patchy and too idiosyncratic to serve students well. Thus there are long and involved calculations of collective scattering and of the growth of clustering from an initially unclustered state; these are difficult to follow and lead neither to astronomically useful results nor to a significant sharpening of physical intuition. On the other hand, the treatment of the structure and instabilities of ellipsoidal and disk-like systems is at best sketchy.

Overall, the selection of material reflects the author's own research interests, and the distribution of emphasis differs somewhat from that typical of modern research in the field. The book's length is a consequence of its enjoyable but discursive style, rather than a reflection of the completeness of its contents. Unfortunately, we shall have to go on waiting for a comprehensive discussion of modern gravitational dynamics.

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Author and augur in theoretical physics

John Stachel

Wolfgang Pauli: Scientific Correspondence with Bohr, Einstein, Heisenberg a.o. Vol. II: 1930–1939. Edited by Karl von Meyenn. Springer-Verlag: 1985. Pp. 783. DM 298, \$112.

LIKE its predecessor (Vol I: 1919–1929), this book is indispensable reading for anyone seriously interested in the history of theoretical physics in this century. In addition to his own very significant theoretical contributions, Wolfgang Pauli was one of the “augurs”, as Einstein called them, whose judgement of new ideas is so important for their acceptance or rejection by the physics community. Indeed, Pauli came more and more to assume the role of the “conscience of physics”, now denied Einstein because of his heretical views on quantum mechanics (Pauli’s nickname — “Zweistein” — bears witness to this transfer of *mana*). “No form of approval could be more precious to physicists, not excluding Bohr, than Pauli’s benevolent nodding” Leon Rosenfeld recalled. Not that Pauli’s judgements were always definitive. In 1931, for example, he wrote to Peierls: “About semiconductors, one shouldn’t work on them, it’s a filthy mess [Schweinerei], who knows if semiconductors even exist” (p.94). More often, however, his views prevailed, even when he opposed other “augurs”. His prompt rejection of Bohr’s attempt to explain the continuous energy spectrum of electrons in β -decay (“The idea of a renunciation of energy conservation in the β -spectrum is and remains in my opinion a cheap and quite clumsy philosophy!”, p.4) helped lead Pauli to his greatest triumph: the prediction of the neutrino.

While the first volume of correspondence centred around discussion of the foundations of non-relativistic quantum mechanics, this collection focuses on relativistic quantum mechanics, quantum field theory and what we now call elementary particle physics. Almost half of the 362 letters are exchanges with Heisenberg. Other major correspondents (over ten letters) include Bohr, Dirac, Ehrenfest, Kemmer, Klein, Peierls and Weisskopf. (There is also a 15-item supplement to Vol. I.) Many of the letters deal with technical developments in the evolving quantum theory of fields and should be read in conjunction with the relevant published papers of Pauli and others. Editorial notes to the letters provide references to the papers; the facsimile edition of Pauli’s *Collected Scientific Papers*, and a similar edition of Heisenberg’s *Gesammelte Werke* now being issued, facilitate such comparisons, though one hopes that in future coordin-

ated editions of writings and correspondence will provide cross-references.

The extensive discussion of Dirac’s hole theory makes the absence of an edition of Dirac’s papers keenly felt. Pauli’s unhappiness with the hole theory seems to have been a driving force behind his attempts to develop a quantum field theory in which pair creation would emerge naturally. When he and Weisskopf were able to provide such a theory for scalar bosons, Pauli referred to it as their “anti-Dirac paper” (p.333). Another area in which Pauli was stimulated by dissatisfaction with Dirac’s work was the theory of higher-spin particles. Working with Fierz, Pauli first believed he had shown that such a theory was impossible for particles of spin greater than one, but then realized how to do it.

The only major discussion of the foundations of quantum mechanics in this volume is in response to Einstein’s challenge, in the well-known Einstein–Podolsky–Rosen (EPR) paper, to the completeness of the description of physical reality offered by the theory. Pauli informed Heisenberg of the paper with a characteristic example of his *politesse*: “Einstein has once again expressed himself publicly on quantum mechanics. As you know, this is a catastrophe every time it happens” (p.402). Yet Pauli immediately grasped the importance of the issue of non-separability raised by the paper: “Quite independently of Einstein . . . in a systematic foundation of quantum mechanics, one should start more from the composition and separation of systems . . . This is indeed—as Einstein has correctly sensed—a very fundamental point . . .” (p.404). Pauli encouraged Heisenberg to reply to the EPR paper, which he did in a previously unpublished manuscript printed on pp. 409–418.

While Pauli functioned well as a “conscience of physics”, he never took over Einstein’s role as “conscience of humanity”. His letters are notably shallow in their references to political events of a decade that spanned the world economic crisis, the advent of fascism in Germany and the crises in the Rhineland, Spain, Austria and Czechoslovakia which led to the outbreak of the Second World War. Pauli is clearly opposed to the rise of fascism, as well as the totalitarian turn taken by the Russian Revolution; but his references to such events primarily concern attempts to mitigate the personal difficulties (for some, like Heisenberg) and tragedies (for others, like Kottler) of fellow physicists or relatives, rather than any broader political perspective. The outbreak of the war caught him by surprise: “I didn’t believe in this war up to the last moment . . .” (p.678). His response is revealing of his sense of obligation and his priorities: “I think it is *my* job to take care for the continuation of the spiritual life in a time like the present one” (p.679).

A sampling of the letters suggests that

there are a large number of misprints, including garbled formulae which make certain letters (for example No. 541) difficult to read. There are also numerous misprints in the annotations, particularly in the transcription of English texts which are sometimes unintentionally humorous (Pauli’s broken arm comes from “slipping on a swimming dock” on p. 85; it is soon moved from a “splint into a cling” on p.92).

A great deal of effort has gone into the editorial annotation, much of it to good purpose. However, some questions must be raised. Detailed information about specific references in a letter is often included in an introductory note. This re-



Star cast — Pauli (top, centre) at the Solvay conference of 1927 with Heisenberg on his left. Bohr and Einstein were also present.

quires frequent footnotes to letters directing the reader back to headnotes. One wonders whether it would not have been better to avoid such annoying interruptions by putting the necessary information into the footnotes in the first place. The sheer quantity of annotation shows a curious decay law. In each of the published volumes, about one-half of the pages in the early part is devoted to introductions and footnotes (since these are printed in smaller type than the main texts, this means the ratio of commentary to text is well over one to one). The number of pages of commentary falls off steadily as the volumes progress, until it reaches about 15 per cent of the total near the end. Did the editor’s enthusiasm slacken, was he under pressure to finish off each volume or is there some other explanation? In any case, the total amount of annotation seems excessive, and much could be pruned without great loss to the reader. An editor is under no obligation to share the fruits of *all* his or her research with the reader. One does not really admire a museum where the descriptions furnished by the staff begin to divert attention from the objects to be studied. □

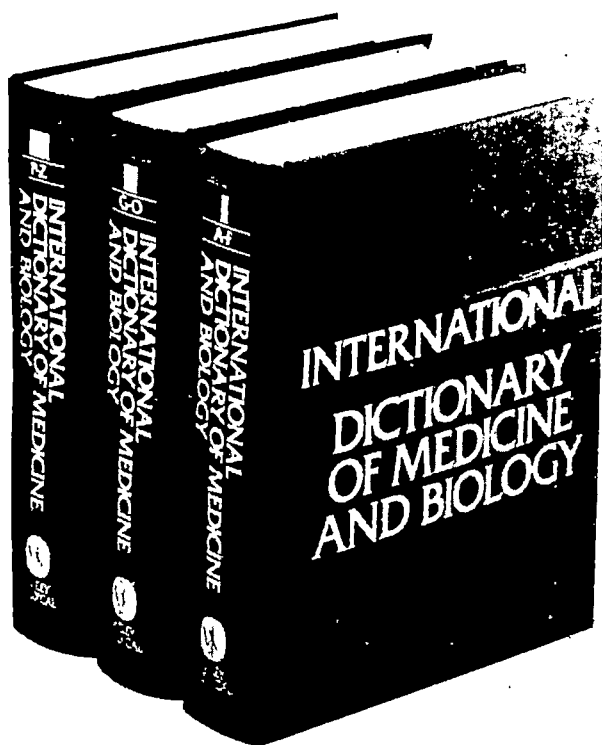
John Stachel, 745 Commonwealth Avenue, Boston, Massachusetts 02215, USA, is Professor of Physics at Boston University, and editor of the *Collected Papers of Albert Einstein*, the first volume of which appears next year.

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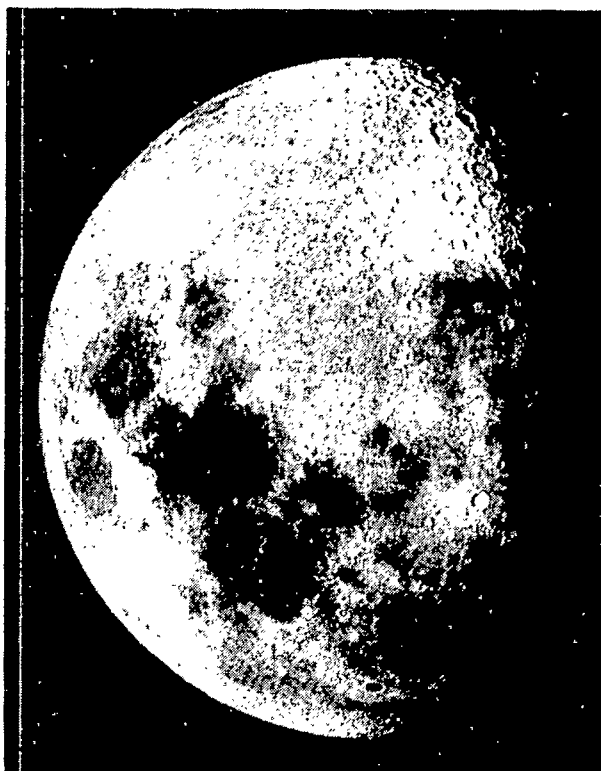
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Hubble's constant determined using very-long baseline interferometry of a supernova

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Determination of the angular expansion velocity of the radiosphere of the supernova SN1979c in the galaxy M100 in the Virgo cluster, combined with determination of the radial expansion velocity of the photosphere, yields an estimate of the distance to the Virgo cluster centre of $D_{\text{Virgo}} = 19^{+8}_{-6}$ Mpc and of the Hubble constant of $H_0 = 65^{+35}_{-25}$ km s⁻¹ Mpc⁻¹, where the uncertainties given are based on conservative, but reasonable estimates of all significant errors and are intended to represent 90% confidence intervals.

THE supernova SN1979c in the galaxy M100 (NGC4321) in the Virgo cluster has been emitting several millijanskies of radio radiation at 5 GHz, starting about 1 year after the explosion¹. Figure 1 displays a radio map of the galaxy; the supernova is situated at the southern edge of a spiral arm. Optical spectral analysis² shows that the radial expansion velocity reached values of the order of 12,000 km s⁻¹. At a distance of, for example, 20 Mpc, this expansion velocity is equivalent to a change in the angular diameter of the exploding star of 0.25 arc ms yr⁻¹. Such a rate of change of the angular diameter of a supernova can be monitored with the radioastronomical technique of very-long-baseline interferometry (VLBI), if the presently most sensitive and most widely spaced antennas are used, and if all of them are equipped with the broad band Mark III VLBI system³.

The determination of a supernova's angular expansion rate, coupled with the determination of its radial expansion rate, allows inference of the distance to the supernova and, with its redshift corrected for local streaming motions, inference of H_0 (ref. 4). This article presents such determinations and inferences from observations of the supernova SN1979c. Preliminary results have been given earlier⁴.

VLBI observations

We made VLBI observations of SN1979c at several epochs after the time of explosion, which we assume to be 1 April 1979, about 19 days before maximum light. The observations, and the abbreviations used below, are summarized in Table 1.

At each station a hydrogen maser frequency standard was used to govern the local-oscillator chain and the 'time tagging' of the recorded data. All observations were made with the most sensitive VLBI system available [Mark III in recording mode A (see ref. 3)]. We used a bandwidth of 56 MHz, except for observations involving station Y, whose intermediate-frequency filters limited the bandwidth to 48 MHz. The data were correlated with the Mark III VLBI processor of the Haystack Observatory, located in Westford, Massachusetts.

Each of the observations of SN1979c was straddled by observations of the calibrator sources OJ287 and/or OQ208. With the clock-epoch and clock-rate information determined from the observations of the calibrator sources, we limited the search for fringes of the supernova to delay and delay-rate ranges that corresponded to windows on the sky with widths as narrow as possible but of at least 1 arc s in extent. The window

centres corresponded to the supernova position determined previously with the VLA⁶. (The positions of many celestial sources determined with the VLA (Very Large Array) agree with those determined by VLBI to within 0.3 arc s; see ref. 5 for comparison of position determinations.) The narrow search windows allowed the reliable detection of far weaker fringes than is possible when the search must extend over many independent delay and delay-rate channels to account for uncertainties in the relative clock epochs and rates (see ref. 7).

When fringes were detected, we used the corresponding information to narrow the search windows even further. In cases where fringes were detected with two two-element interferometers of a triplet of antennas, closure observables were calculated for the third two-element interferometer. The values of these observables have a probability of ~1% of being due to noise alone. For observations of SN1979c on 1 December 1983, these procedures allowed us to quadruple the number of detections relative to the number obtainable with 'normal' processing. From observations on 9 May 1983, at 8.4 GHz, we detected the source only with the BM interferometer. For the DM interferometer, we determined a 3σ upper limit ($\sigma \equiv 1$ statistical standard deviation) for the correlation coefficient, a value that corresponds to a probability of 10% that noise somewhere in the search area of 0.3×0.6 arc s would exceed this upper limit. The other upper limits were ignored in the further analysis, as they were too high to be of use.

The correlation coefficients were corrected for any coherence losses and for the bias arising from the fact that they are always positive⁸, and were calibrated to yield correlated flux densities⁹.

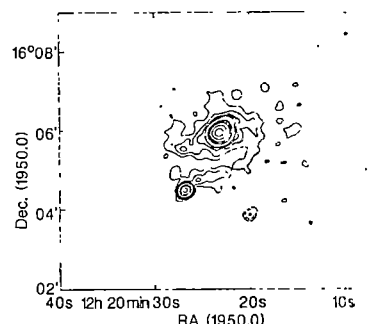


Fig. 1 Radio map of the galaxy M100 showing the supernova SN1979c [$\alpha(1950.0) = 12^{\text{h}} 20^{\text{m}} 26.71^{\text{s}}$, $\delta(1950.0) = 16^{\circ} 04' 29.5''$] situated at the southern edge of one of the galaxy's spiral arms. The contour levels are at 2.5, 5.0, 7.5, 10, 20, 30, 50, and 80% of the peak flux density per beam area of 9.75 mJy.

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Table 1 Observations of SN1979c

Date of observation	Radio frequency (GHz)	Polarization*	Antennas†	MSIA‡	Sensitivity§ of MSIA (7 σ) (mJy)	No. of scans with MSIA
8 December 1982	5.0	LCP	BGOY	BY	1.7	4
9 May 1983	2.3	RCP	BDFG(K)MO(S)	DM	1.5	2
9 May 1983	8.4	RCP	B(DK)M	DM	2.1	2
1 December 1983	5.0	LCP	BG(K)OY	BY	1.7	7
31 May 1984	1.7	LCP	BGOY	BY	1.4	5

According to Weiler *et al.*⁶, the coordinates of SN1979c are: α (1950.0) = 12 h 20 min 26.71 s, δ (1950.0) = 16°04'29".5.

* LCP, Left-hand circular polarization. RCP, Right-hand circular polarization.

† B, 100-m antenna in Effelsberg near Bonn, FRG; belongs to the Max-Planck-Institut für Radioastronomie. We give for each antenna a representative value of the product, S_{sys} , of system temperature and inverse sensitivity in Jy for each of the frequencies in GHz: S_{sys} (1.7, 2.3, 5.0, 8.4) = 28, 260, 47, 150. D, 64-m antenna in Goldstone, California, USA; belongs to NASA. S_{sys} (2.3, 8.4) = 29, 45. F, 25-m antenna in Ft Davis, Texas, USA; belongs to Harvard College Observatory. S_{sys} (2.3) = 1,650. G, 43-m antenna in Green Bank, West Virginia, USA; belongs to the National Radio Astronomy Observatory. S_{sys} (1.7, 2.3, 5.0) = 66, 590, 190. K, 37-m antenna in Westford, Massachusetts, USA; belongs to the Northeast Radio Observatory Corporation. S_{sys} (2.3, 5.0, 8.4) = 5900, 650, 800. M, 64-m antenna near Madrid, Spain; belongs to NASA. S_{sys} (2.3, 8.4) = 36, 50. O, 40-m antenna near Big Pine, California, USA; belongs to California Institute of Technology. S_{sys} (1.7, 2.3, 5.0) = 235, 425, 300. S, 25-m antenna in Onsala, Sweden; belongs to Onsala Space Observatory. S_{sys} (2.3) = 1,000. Y, Equivalent to a ~130-m antenna (~26 × 25 m antennas of the VLA 'phased up') near Socorro, New Mexico, USA; belongs to the National Radio Astronomy Observatory. S_{sys} (1.7, 5.0) = 26, 27. All two-element interferometers with the symbol for at least one antenna in parentheses failed to yield fringes.

‡ Most sensitive two-element interferometer of array.

§ Maximum sensitivity for coherent integration of 12 min and a bandwidth of 50 MHz. Here and hereafter, 1 σ denotes one statistical standard deviation.

|| One scan consists of an observing time of ~12 min using a bandwidth of ~50 MHz.

Table 2 Parameter estimates for SN1979c

Date of observation	Time since explosion* (yr)	Frequency (GHz)	$S_{\text{tot}}^{\text{VLA}†}$ (mJy)	S_{tot} (mJy)	Angular diameter‡ (mas)
8 December 1982	3.691	4.99	5.5 ± 0.3	5.30 ^{+0.26} _{-0.54}	1.05 ^{+0.09} _{-0.10}
9 May 1983	4.117	2.29	—	9.7 ^{+0.8} _{-0.4}	1.67 ^{+0.26} _{-0.12}
9 May 1983	4.117	8.42	3.9 ± 0.2	3.8 ^{+0.2} _{-0.2}	>0.91
1 December 1983	4.672	4.99	4.2 ± 0.2	4.08 ^{+0.45} _{-0.38}	1.43 ^{+0.07} _{-0.07}
31 May 1984	5.173	1.67	7.9 ± 0.6	7.75 ^{+0.61} _{-0.63}	2.16 ^{+0.44} _{-0.50}

* The assumed date of explosion is 1 April 1979.

† The total flux density of the supernova measured with the VLA alone. For the 5-GHz data, the errors were taken to be 5% of the total flux density so as to include both statistical standard deviations and possible systematic errors. For the 1.7-GHz data, the error represents the variation of the total flux densities determined from observations of the source on 31 May 1984 and 1 June. In all four cases, the 1 σ noise level in apparently blank fields of the maps near the position of the supernova is more than three times smaller.

‡ Estimated angular diameter for a uniform sphere model of the brightness distribution of the supernova. For the meaning of the errors, see text.

The calibration procedure was checked for our observations of the calibrator sources, which have relatively simple brightness distributions, and was found to yield flux densities consistent with the predictions from a 'best-fit' circular gaussian brightness distribution to within $\pm 7\%$ in each case.

Angular diameter determinations

The angular diameters of the supernova at the epochs of our observations were all small compared with the smallest fringe spacing obtainable with our arrays. Hence, the radio brightness distribution of the supernova cannot be well determined from our VLBI data. Further, because of the low declination of SN1979c and the mainly east-west orientation of our VLBI arrays, the highest resolution in the north-south direction was about four-fold lower than that in the east-west direction, making any (small) deviations of the brightness distribution from circular symmetry difficult to discern.

Under these circumstances, any angular diameter of the supernova inferred from the VLBI data will be strongly model-dependent. We, therefore, considered a range of plausible possibilities, assuming that any given model, if applicable at all, would be applicable for all of our epochs of observation. Clearly, a model in which the brightness is distributed in a thin circular ring, normal to our line-of-sight, will lead to the smallest inferred angular diameter. By contrast, a model in which the brightness is highly concentrated near the centre will yield a relatively large value for the inferred angular diameter.

We take as an example an optically-thin uniform-sphere

model. From the correlated flux densities, S_{corr} , from each epoch of observation, we estimated by weighted-least-squares the total flux density, S_{tot} , and the angular diameter, θ_{us} , as well as the standard errors in these estimates implied by the statistical standard errors of S_{corr} . The values of S_{corr} were normalized by dividing by S_{tot} and plotted together with the predictions of the model in Fig. 2.

We estimated crudely the contribution of possible calibration errors to the uncertainties of the parameter estimates by changing, in turn, the sensitivity of each antenna of the array by $\pm 15\%$ while keeping the sensitivities of the other antennas fixed. We consider this choice of the value for the uncertainty of the antenna sensitivities to be conservative in view of our check of the calibration procedures for the calibrator sources, mentioned above. The largest variations in the estimated parameters due to the changes of the sensitivities of the antennas were twice as large as the corresponding statistical standard deviations. For the uncertainty of each parameter estimate, we adopted the root-sum-square (r.s.s.) of the value of the largest variation and the statistical standard deviation. The results of the analysis are given in Table 2.

To check the sensitivity of our results to the choice of antennas, we repeated the analysis with data from subsets of antennas. The estimated parameters from this analysis were reasonably consistent with the parameter values obtained from the analysis of all the data⁴.

We also tried to check for any significant deviation from circular symmetry by using elliptical gaussian models. For each

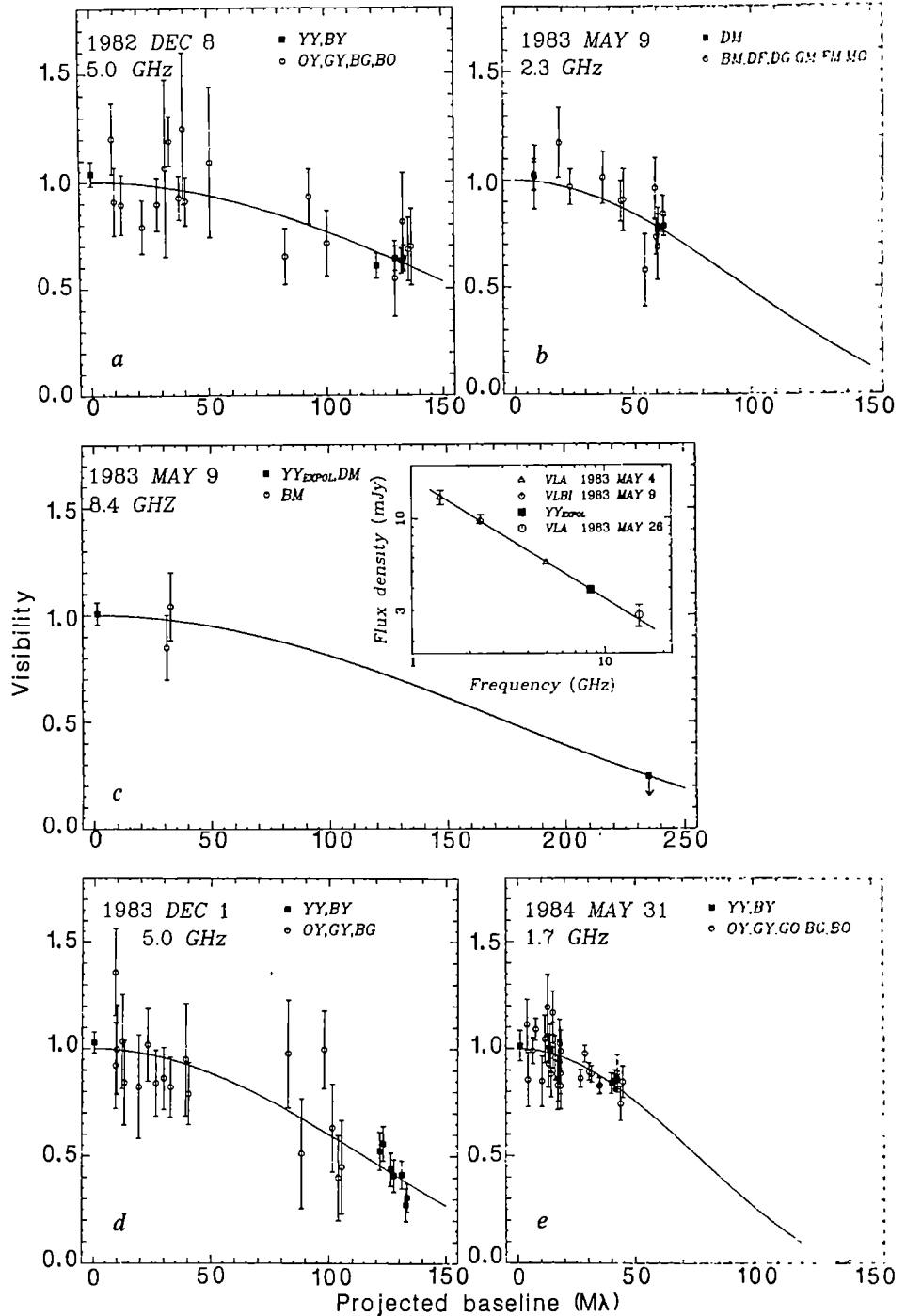


Fig. 2 *a-e*, Measured visibility amplitudes and predictions from the fit of a uniform sphere model. The filled squares show the most significant data points. Antenna pairs are listed in order of increasing baseline length. The symbol YY indicates the values for the total flux density measured with the VLA. The inset of Fig. 2*c* shows the weighted-least-squares fit to the spectral index, $\alpha = -0.69 \pm 0.06$ ($S_\nu \propto \nu^\alpha$), from the values of the total flux density at 1.5 and 5 GHz obtained at the VLA (ref. 1) and at 2.3 GHz obtained with our VLBI array 5 days later. The filled square shows the value for the flux density extrapolated with this power law to $\nu = 8.42$ GHz. This value is used as the zero-baseline correlated flux density (YY_{EXPOL}) in Fig. 2*c*. Also shown is the data point at 15 GHz obtained with the VLA (ref. 1) ~ 3 weeks later.

set of 5-GHz observations of the supernova, the ratio of the estimates of the major and minor axes was unity to within 1σ ; however, 1σ for these ratios was as large as ~ 2.5 . A higher north-south angular resolution is needed to determine, or to derive a useful upper bound on, any deviation from circular symmetry the source might have.

We also computed closure phases to search for evidence for asymmetry of the radio structure of the supernova. However, the closure phases were randomly scattered, thereby indicating no significant departure from zero. This result is also consistent with, but not conclusive evidence for, the supernova being circularly symmetric.

For a circular-gaussian model for the brightness distribution, the angular diameter at half maximum is 1.81 times smaller than θ_{us} , and at 10% of maximum about equal to θ_{us} . A thin ring, as stated above, gives the smallest diameter, 1.65 times smaller than θ_{us} . Optically thin shells give diameter determinations intermediate between those of a thin ring and a uniform sphere;

for example, for a uniform shell of thickness 5–25% of the outer radius of the shell, the diameter becomes, respectively, 1.34–1.24 times smaller than θ_{us} (see refs 10, 11).

An optically-thick shell or sphere, which is equivalent to a uniform disk, yields a diameter determination $\sim 10\%$ smaller than θ_{us} . However, since the time of our first VLBI observations, the radio spectrum of the supernova has had a spectral index, $\alpha(S_\nu \propto \nu^\alpha)$ of ~ -0.6 (ref. 12), indicating that the radio source is optically thin. Optically thick sources can therefore be ignored, but in any event do not extend the uncertainty of our diameter estimates.

An optically-thin shell of emission is favoured by Chevalier¹³, who assumes the radio radiation is synchrotron emission from the interaction of the supernova shock front with a circumstellar medium created by presupernova mass loss. The classical supernova remnant with a shell of emission is Cas A (see, for example, ref. 14). With $\sim 80\%$ of all supernova remnants (SNRs) being conventionally represented as shell-type models¹⁵, the shell

geometry seems to be the standard for the radio emission for epochs at least several hundred years after the supernova explosion.

An optically-thin uniform sphere of emission, similar to a centre-filled remnant, that is, a plerion, could be expected from one type¹⁶ of centrally driven models (refs 16, 17; see also ref. 18). The classical SNR of plerionic morphology is the Crab nebula. Only a few such examples have yet been found¹⁵. Moreover, plerionic SNRs have flatter spectra ($\alpha > -0.3$) than shell-like SNRs, which have $\alpha \sim -0.45$ (ref. 15), and thus may be less closely related to SN1979c than shell-like SNRs, if evolutionary effects on the geometry and the spectrum of a remnant do not make such comparisons irrelevant.

A ring of emission for SNRs has recently been suggested by Manchester¹⁹, who assumes interaction of a rotating pulsar beam of particles with the medium close to the boundary of the SN ejecta. We include such a geometry in estimating the uncertainty of our diameter determinations of SN1979c.

Considering all of these possibilities, we conclude that the diameters inferred from the uniform sphere and the thin ring probably straddle the actual diameter, which is likely to be that of an optically-thin shell.

Time dependence

The values for the angular diameters, θ , of the supernova for both the uniform sphere and the ring models are plotted in Fig. 3 as a function of time. The results from the 5-GHz data indicate that, with high probability, the supernova is expanding. These results, combined with the zero diameter at the time of explosion, allow the deceleration (or acceleration) to be estimated from an assumed time dependence of the angular diameter: $\theta \propto t^m$ (see refs 20, 21). Taking the standard errors for the angular diameter determinations to be independent yields the result $m = 1.3 \pm 0.5$.

Deceleration of the expansion of the supernova would be expected for the shock-front model, and evidence for it has been found in shell-type SNRs (see ref. 22). In contrast, acceleration of the expansion would be expected for the centrally-driven model and some evidence for weak acceleration has been found in the Crab nebula²³. However, neither the accuracy nor the number of our measurements is yet sufficient to discriminate between these models on the basis of our estimate of the parameter m . More observations at later epochs are required to determine with useful confidence any deviation of m from unity.

Frequency dependence

The value we obtained for the angular diameter at 2.3 GHz is significantly larger than the value for the diameter at 5.0 GHz when compared at the same epoch (we assume $m = 1$). The value at 1.7 GHz, because of its relatively large uncertainty, and the lower bound at 8.4 GHz, are both consistent with the diameter's not being a function of frequency.

Is this frequency dependence inherent to the supernova or could the angular diameter be broadened noticeably by scattering in the propagation medium? From a survey of pulsar interstellar scintillation, Cordes *et al.*²⁴ predict that the scattering angle, θ_{scat} (FWHM of a gaussian), of a source at the supernova's galactic latitude, $|b^{\text{II}}| = 77^\circ$, is between 170 and 340 arc μs for 1.7 GHz and scales as $\theta_{\text{scat}} \propto \nu^{-2.2}$. To calculate the effect of θ_{scat} on the estimate of θ_{us} , we multiply θ_{scat} by 1.81 to convert its value to that of the 'equivalent' uniform sphere. Thus, we could, at $\nu \geq 5$ GHz, essentially exclude any broadening of the angular diameter and at $\nu \sim 2.3$ and 1.7 GHz expect broadening of no more than 2 and 4%, respectively. However, if we use weighted least squares to fit to the observed data a function of the form, $\theta_{\text{us}}^2(\nu, t) = 1.81^2 \theta_{\text{scat}}^2(\nu) + \theta_{\text{us}}^2(t)$, with $\theta_{\text{scat}} \propto \nu^{-2}$ and $\theta_{\text{us}} \propto t$ as plotted in Fig. 3, we obtain a good fit and a value for θ_{scat} (1.7 GHz) of 1.1 arc ms, which is about three-fold larger than the above upper bound of Cordes *et al.* In fact, VLBI determinations by Resch²⁵ at 74, 111, and 196 MHz of angular diameters of extragalactic sources, situated within $\sim 20^\circ$ of SN1979c indicate $\theta_{\text{scat}} \propto \nu^{-n}$ with $n < 2$ and values for θ_{scat} much larger than

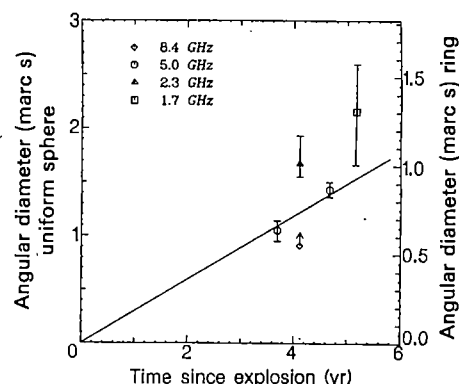


Fig. 3 The angular diameter determinations assuming two (extreme) models of the brightness distribution. The solid straight line represents a least-squares fit to the assumed zero-point origin and the two 5-GHz values. The 1.7 and 2.3-GHz values are probably subject to broadening by scattering in the propagation medium (see text).

1 arc ms when extrapolated to 1.7 GHz. Also, as Cordes *et al.* noted, some values of Resch exceeded by up to a factor of 1.6 their upper bounds even when a scattering law of $n = 2.2$ was assumed. We thus conclude that our estimate of θ_{scat} is within the range of others' estimates and that our diameter determinations at 2.3 and 1.7 GHz could have been significantly broadened by scattering in the propagation medium, but that our determinations at 5.0 and our lower bound at 8.4 GHz could not. If the angular diameter at the lower frequencies is indeed broadened by scattering, the difference between the diameter determinations at different frequencies should decrease as the source expands; future observations at more than one (low) frequency could help to resolve this issue.

Distance to M100 and Hubble's constant

To estimate the distance to SN1979c, we combine our radio determination of the supernova's angular expansion velocity with optical determinations of the radial expansion velocity. We first list the main assumptions used in making this estimate: (1) the widths of optical spectral lines reflect expansion of the supernova's photosphere; (2) the expansions of the photosphere and the radiosphere are each isotropic within a factor $\mu = 1.0 \pm 0.15$; (3) the expansion velocity of the radiosphere equals that of the outer boundary of the supernova's shock front and is, for at least 1 yr after the explosion, a factor of $\eta = 1.2 \pm 0.2$ greater than the expansion velocity of the photosphere; and (4) the angular diameter θ of the radiosphere can be described by $\theta \propto t^m$, with t the time since explosion and m constrained by $m \leq 1$ (no acceleration allowed).

The first assumption is based on the finding that in the early stages of supernova evolution the time dependences of the inferred photospheric temperature and the received flux are both consistent with an expanding black body (see, for example, ref. 26). However, to some extent, the broadening of the lines may also be caused by other processes, for example, turbulence in the emission region.

The second assumption is supported by observations that most young and medium aged SNRs are approximately circularly shaped (ref. 27 and refs therein) and by the likelihood that the degree of circular symmetry for supernovae is even higher. In the future, for the photosphere, optical spectropolarimetry might reveal any anisotropic expansion at least in the early stages of a supernova's evolution^{28,29}. For the radiosphere, any deviations from non-isotropic expansion in the plane of the sky could, in principle, be determined accurately through VLBI by mapping the brightness distribution of the supernova, but our present data cannot yield useful determinations. Our assumed uncertainty for μ is consistent with observations of most SNRs and with inferences for supernovae.

The third assumption is based on the fact that charged particles are accelerated in shock front regions and thus are likely

to radiate radio waves. In the shock front model, the radiosphere is a shell of emission with its outer radius being equal to the outer radius of the supernova's shock front and its inner radius equal to the radius of the (H α) photosphere³⁰. In centrally driven models, the radio radiation emanates from the shock front¹⁷ or predominantly from inside the shock front shell¹⁶. For the latter case, the photospheric material is assumed to have developed a filamentary structure very soon after the explosion as otherwise the opacity of the material would prevent penetration of radio radiation³¹. We do not discuss here the likelihood of such a process; rather we assume that any emission region inside the shock front shell is not concentrated near the center but is described by a uniform sphere with its expansion velocity linked to that of the photosphere such that $\eta \geq 1$.

What is the expansion velocity of the photosphere? Optical and ultraviolet spectra of SN1979c were obtained at several epochs^{2,32-35}. The dominant profile in the optical spectrum is the H α emission line. The blue extension of this line indicates expansion velocities of (ref. 2) $\sim 10,500 \text{ km s}^{-1}$ on 28 May, 26 June and 19 November 1979, and (ref. 35) $\sim 9,000 \text{ km s}^{-1}$ on 10 April 1980. The minima of the absorption troughs of the P Cygni profiles of the ultraviolet and optical lines other than H α indicate expansion velocities between $\sim 8,800$ and $10,000 \text{ km s}^{-1}$ in the period between 22 April and 1 July 1979 (refs 2, 33). According to Kirshner³⁵, all these values define the expansion velocity of the photosphere; the maximum expansion velocity of supernova ejecta is larger. The blue edge of the absorption trough in the He I $\lambda 5,876/\text{Na I D}$ profile, in spectra taken between 22 April and 26 June 1979, reveals a velocity of $\sim 12,000 \text{ km s}^{-1}$ (ref. 2); this value probably defines the expansion velocity of the outer boundary of the supernova's shock front. Considering these various results, we adopt a value of $v_{\text{ph}} = (9 \pm 1) \times 10^3 \text{ km s}^{-1}$ for the expansion velocity of the photosphere for an epoch, say, 1 yr following the explosion, and a value of $\eta v_{\text{ph}} = (11 \pm 2) \times 10^3 \text{ km s}^{-1}$ for the expansion velocity of the radiosphere at this epoch, consistent with the maximum expansion velocity of the supernova ejecta (for $0.8 \leq m \leq 1.0$; see below).

The fourth assumption allows for the expansion velocity of the shock front to vary with time. The parameter m was estimated to be ≥ 0.89 from the change of the width of H α ³⁶, and ≥ 0.90 from the radio light curve³⁷, both values consistent with the inference from our VLBI observations. More VLBI observations are needed not only to determine m more accurately, but also to test our assumption that a simple power law represents adequately the expansion of the radiosphere. In fact, the range of values of m consistent with the present VLBI observations is so great as to be in large part inconsistent with assumptions (3) and (4), when the latter are coupled with the estimates, discussed above, of expansion velocities inferred from optical spectra.

Using the (assumed) relation between the velocities of expansion of the radiosphere and the photosphere at an epoch, t_{ph} , 1 yr after the supernova explosion, to determine the proportionality constant in the (assumed) relation $\theta \propto t^m$, and the relation $\theta_{\text{ph}} = 2v_{\text{ph}}/D$, where θ_{ph} is the angular velocity of expansion of the photosphere at that epoch, v_{ph} the corresponding (radial) expansion velocity, and D our distance from the supernova, we obtain by simple algebra:

$$D = \frac{2\mu\eta v_{\text{ph}} t}{\theta m} \left[\frac{t}{t_{\text{ph}}} \right]^{m-1}$$

With $\eta v_{\text{ph}} = (11 \pm 2) \times 10^3 \text{ km s}^{-1}$ on, say, 10 April 1980 (that is, $t_{\text{ph}} = 3.2 \times 10^7 \text{ s}$), with m restricted to $m = 0.9 \pm 0.1$, and with $\theta = 1.0 \pm 0.25 \text{ arc ms}$ (see Fig. 3) at $t = 1.35 \times 10^8 \text{ s}$, the epoch midway between our two 5-GHz data points, we obtain for SN 1979c: $D_{\text{SN1979c}} = 19^{+8}_{-6} \text{ Mpc}$. If we further assume that SN 1979c is in M100 (Fig. 1) and that the distance to M100 is within 10% of the distance D_{Virgo} to the centre of the Virgo cluster, since M100 is within 6° of this centre and has a redshift consistent with this location, we get:

$$D_{\text{Virgo}} = 19^{+8}_{-6} \text{ Mpc}$$

The range of uncertainty given is intended to represent the 90% confidence interval and is based on the assumption that each of the parameters in the above expression for D contributes independently.

A value of Hubble's constant, H_0 , can be obtained by combining our distance estimate with determinations of the mean (radial) velocity, v_0 , of Virgo away from the centre of the Local Group and the correction for the infall (radial) velocity, v_{infall} , of the centre of the Local Group towards Virgo: $H_0 = (v_0 + v_{\text{infall}})/D_{\text{Virgo}}$. Unfortunately, $v_0 + v_{\text{infall}}$ is not well determined. Considering the spread in the various published values for v_{infall} , based on observations of galaxies (see refs 38-43), as well as the equivalent values estimated from the anisotropy of the microwave background⁴⁴⁻⁴⁶, and the different estimates of v_0 (see refs 47-49), we take $v_0 + v_{\text{infall}} = 1,250 \pm 150 \text{ km s}^{-1}$ and obtain

$$H_0 = 65^{+35}_{-25} \text{ km s}^{-1} \text{ Mpc}^{-1}$$

where the range of uncertainty given is dominated by that for D_{Virgo} and is also intended to represent the 90% confidence interval.

Comparing our estimates with those obtained by de Vaucouleurs⁵⁰ ($D = 11.8 \text{ Mpc}$ for M100, equivalent to $H_0 \sim 100 \text{ km s}^{-1} \text{ Mpc}^{-1}$) and by Sandage and Tammann^{51,52} ($D = 22.2 \text{ Mpc}$ for M100, equivalent to $H_0 \sim 50 \text{ km s}^{-1} \text{ Mpc}^{-1}$), we find that our estimates are nearly midway between theirs but hardly distinguish between them.

More, and more accurate, VLBI measurements on SN1979c and other supernovae should allow us to improve the determinations of θ , m , and μ . However, we have not yet conceived of any purely observational means to relate the photosphere and the radiosphere. The uncertainty in this relation will therefore likely dominate the uncertainty in any estimate of H_0 based on the method described here, until the relation can be reliably modeled or until optical interferometry, perhaps from earth orbit, can be used to measure directly the angular size of a supernova's photosphere.

Conclusion

VLBI observations of SN1979c in the galaxy M100 yielded estimates of the angular size of the supernova. These measurements were used, together with other information, especially from the optical spectra, to estimate the supernova's distance and Hubble's constant. The range of the uncertainties of our estimates can probably be decreased with new and more sensitive VLBI observations of SN1979c in the near future. The sensitivity can be increased by recording the data on longer tapes, thus allowing longer integration times. It can also be increased by including in the array the 305-m diameter antenna at Arecibo. This antenna's low latitude would increase our north-south resolution two-fold and might enable us either to determine the brightness distribution of SN1979c or, at least, to measure, or place useful bounds on, its deviation from circular symmetry.

Observations of SN1979c at 5 GHz at a third epoch would probably be useful for a more accurate determination of the deceleration (or, possibly, acceleration) of the expansion. More observations at lower frequencies would allow more detailed investigation of whether the apparent source diameter is broadened by scattering in the propagation medium.

About one supernova per year should be detectable with our present VLBI system. Moreover, the Hubble Space Telescope should allow measurement of supernova spectra at the same epochs at which VLBI techniques could be used to measure the angular size of the supernova. Combining such measurements should enable us to determine distances to galaxies as far away as $\sim 40 \text{ Mpc}$. Observing supernovae in many different directions will allow us to study statistically the deviations of their (radial) velocities from the Hubble flow and thereby provide an indication of the accuracy of our inferred value of H_0 .

Our method is the fourth that makes use of supernovae as distance indicators (for descriptions of the other three, see refs 53-56). Each has its difficulties, but each may be useful for deriving distance estimates at least partially independent of the

others and thereby for allowing useful redundancy in the estimates of Hubble's constant.

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Magnetic confinement of a neutral gas

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A three-dimensional dynamic trap for confining a collisional neutral gas is described. The trap uses the interaction of the magnetic moments of the gas atoms with a time-dependent magnetic field. Collisions lead to the evaporative cooling of the trapped atoms and to the viscous heating of the gas, particularly at high densities. This magnetic trap may allow the attainment of Bose-Einstein condensation in spin-polarized atomic hydrogen gas.

THE dynamic magnetic confinement of a neutral gas by the interaction of the magnetic moments of the gas atoms with a magnetic field including a rapidly oscillating component creates a magnetic trap which may provide a means of producing a Bose-Einstein condensation in a spin-polarized atomic hydrogen gas.

Fritz London¹ was the first to consider the phenomena of superconductivity in metals and superfluidity in liquid ⁴He in terms of quantum mechanics on a macroscopic scale. Since then, superfluidity has been found to occur in liquid ³He below 3 mK (ref. 2), and it is thought to occur in neutron stars³. The recently achieved stabilization of spin-polarized atomic hydrogen gas in strong magnetic fields (9 T) and at low temperatures (100-500 mK) presents a possible new opportunity to observe macroscopic quantum phenomena^{4,5}. Because hydrogen atoms behave as bosons, the macroscopic manifestations of Bose-Einstein condensation are expected to lead to a variety of interesting

quantum properties in this dilute, weakly interacting Bose-Einstein gas. The occurrence of Bose-Einstein condensation in an ideal Bose gas is governed by the equation

$$T_c = [h^2 / (2\pi m k_B)] (n / 2.61)^{2/3}$$

where T_c is the critical temperature, n is the particle density, m is the particle mass, h is Planck's constant, and k_B is Boltzmann's constant. Sufficiently high densities and low temperatures must be attained in order to have a Bose-Einstein condensation. Table 1a shows densities and temperatures corresponding to the onset of Bose-Einstein condensation.

Spin-polarized atomic hydrogen gas is formed by collecting atoms in the two low-energy hyperfine Zeeman states (the 'a' and 'b' states, where the electron spin is antiparallel to the magnetic field) in a strong magnetic field at low temperatures⁶. Atoms in these two states are forced towards regions of stronger magnetic field, while atoms in the two high-energy hyperfine states (the 'c' and 'd' states, where the electron spin is parallel to the magnetic field) are forced towards low-field regions. The 'a' state is a mixed state whose wavefunction includes a small

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Table 1 Conditions for Bose-Einstein condensation (a) and parameters for a dynamic trap (b)

a	T_c (K) = 1	0.1	0.01	0.001	0.0001
	n (cm ⁻³) $\approx 5 \times 10^{20}$	1.5×10^{19}	5×10^{17}	1.5×10^{16}	5×10^{14}
b	Temperature T (mK)	Coil radius R (cm)	a.c. current min I (kA-turns)	a.c. field b_z (O) (T)	Frequency min f_e (kHz)
	3	1	15.4	0.97	3.3
	1	1	5.1	0.32	1.9
	0.3	1	1.5	0.09	1
	3	0.5	7.7	0.97	6.6
	1	0.5	2.6	0.32	3.8
	0.3	0.5	0.75	0.09	2

admixture of the electronic spin-up state causing recombination of atoms to molecules in three-body, singlet interaction collisions (where the helium-coated surfaces of the confinement vessel may play the role of the third body). As a consequence, the sample loses its 'a' state population as time progresses, leaving only the pure 'b' state atoms whose nuclei and electrons are polarized parallel to one another (and therefore, have a triplet interaction). Because of the long longitudinal relaxation time (T_1) between the 'b' and the 'a' states, the resulting doubly polarized sample would be relatively long lived^{7,8}, and thus ideal to produce the high-density sample needed to fulfill conditions for Bose-Einstein condensation were it not for the three-body dipole recombination which becomes important at densities above 10^{18} cm⁻³ (refs 9-14). One possible solution is to obtain Bose-Einstein condensation at temperatures below 10 mK, where the required gas phase densities are less than 10^{17} cm⁻³. Unfortunately, at these temperatures surface densities are high even for liquid helium film surfaces (binding energy ~ 1 K), so that surface three-body dipole recombination destroys the gas rapidly⁹⁻¹⁴.

An alternative approach is to remove the influence of surfaces by constructing a three-dimensional magnetic trap¹⁵⁻¹⁷. Static magnetic field minimum traps are possible, but only for the 'c' and 'd' state atoms, which are low-field seekers¹⁵. One could inject a 'b' state gas and use electron spin resonance to flip the electron spins so that the resulting 'c' state atoms would be magnetically confined. However, dipole-dipole interactions are predicted⁹ to cause a rapid flipping of the electron spins and this would lead to a rather rapid loss of the sample. (Note that a variety of three-dimensional electromagnetic traps have been used for the collisionless confinement of small numbers of ions¹⁸.)

Here we investigate a three-dimensional dynamic magnetic trap, where the magnetic field includes a 'fast' time-dependent component. The dynamic trap must provide containment in the presence of frequent collisions. (Thus, one can rule out confinement in a rotating magnetic mirror field where the possible stable single-particle motion is analogous to that of the Trojan asteroids¹⁹.) A simple dynamic trap which is viable in the presence of collisions can be made by passing an alternating current through a circular wire loop located with its axis parallel to a strong uniform static magnetic field. This magnetic field oscillates between a mirror and an anti-mirror configuration and provides containment for the high-field-seeking 'a' and 'b' state atoms (as well as for the 'c' and 'd' state atoms). Here we first analyse the collisionless confinement of a single atom, then investigate the influence of collisions using the classical Boltzmann transport equation and a Monte-Carlo code for the numerical evaluation of particle orbits including collisions. (A quantum-mechanical, mean-field treatment of a collisional gas confined in a dynamic trap²⁰ confirms the classical treatment for temperatures above T_c .) Next, a treatment is given of the evaporative loss of atoms and heat from the trapped gas, and of the viscous heating of the gas. A method is described for filling a dynamic trap using the evaporative cooling which can be enhanced by gradual reduction in the depth of the magnetic well. This may allow the attainment of the conditions for Bose-

Einstein condensation. An alternative approach to Bose-Einstein condensation using a magnetic field and evaporative cooling has recently been proposed by Hess²¹. Finally, we discuss some of the anticipated problems of filling the trap and of observing the Bose-Einstein condensation in a spin-polarized atomic hydrogen gas.

Single-particle motion

The envisaged temporal variations of the magnetic field $\mathbf{B}(\mathbf{x}, t)$ are very much slower than the electron spin precession period. Thus, the potential describing the interaction of the atom with the magnetic field is

$$V(\mathbf{x}, t) = -\mu|\mathbf{B}(\mathbf{x}, t)|$$

where μ is the magnetic moment of the atom. For the case of spin-polarized atomic hydrogen, $\mu \approx \hbar e/(2m_e c) \approx 0.93 \times 10^{-20}$ erg G⁻¹ (or 0.93×10^{-23} J T⁻¹), where m_e is the electron mass. Note that the magnetic moment is parallel to \mathbf{B} for the 'a' and 'b' states of atomic hydrogen so that $\mu > 0$. For the 'c' and 'd' states it is antiparallel and $\mu = -|\mu|$. In the absence of collisions, the equation of motion for an atom of mass m is

$$m \frac{d^2 \mathbf{x}}{dt^2} = -\nabla V \quad (1)$$

In a static field $\mathbf{B}(\mathbf{x})$ a spin-polarized atom with μ parallel to \mathbf{B} (the 'a' or 'b' states of hydrogen) tends to move towards regions of maximum $|\mathbf{B}|$. There are, of course, no isolated maxima of $|\mathbf{B}|$ in free space (Earnshaw's theorem; see, for example, ref. 22).

The magnetic field $\mathbf{B}(\mathbf{x}, t)$ is considered to consist of a uniform, time-independent component $B_0 \hat{z}$ (with $B_0 > 0$), and the time-dependent field $\mathbf{b}(\mathbf{x}, t)$ of one or more circular wire loops with axes coinciding with the z -axis. We consider the case of a single wire loop of radius R . We assume $b^2 \ll B_0^2$ so that $|\mathbf{B}| \approx B_0 + b_z$. In the vicinity of the centre of the loop, we have

$$b_z = b_0(t) \left\{ 1 + \frac{3}{4} \left(\frac{r}{R} \right)^2 - \frac{3}{2} \left(\frac{z}{R} \right)^2 + \dots \right\}$$

where $r^2 = x^2 + y^2$, $b_0(t) = 2\pi I(t)/(cR)$ in gaussian units [or $\mu_0 I/(2R)$ in MKS units], and I is the current in the wire loop. For $b_0 < 0$ the field configuration corresponds to a mirror field, whereas for $b_0 > 0$ it is an anti-mirror configuration. The Taylor expansion simplifies the immediate discussion; the exact field of a circular loop is used subsequently. From equation (1),

$$\frac{d^2 x}{dt^2} = -\alpha(t)x, \quad \frac{d^2 y}{dt^2} = -\alpha(t)y, \quad \frac{d^2 z}{dt^2} = 2\alpha(t)z \quad (2)$$

where $\alpha(t) \equiv (3/2) [\mu b_0(t)/(mR^2)]$. Evidently, the sign of μ is irrelevant if $b_0(t)$ is an oscillatory function with an average value of zero.

Exact solutions to equations (2) exist for the case where $b_0(t)$ or $\alpha(t)$ has a square wave dependence, that is, $\alpha(t) = \alpha_0 \text{sign}[\sin(\omega_e t)]$, where α_0 and ω_e are constants. This case is evidently the temporal analogue of an alternating-gradient or

strong focusing synchrotron²³. The motion in the x, y or z directions can be written down explicitly during the intervals in which $\alpha(t)$ is positive or negative. Thus, the phase space location at some time t for, say, the z motion, $[z(t), \dot{z}(t)/(2\alpha_0)^{1/2}]$, can be expressed as a product of matrices multiplying the initial location $[z(0), \dot{z}(0)/(2\alpha_0)^{1/2}]$. For each interval with $\alpha < 0$ or $\alpha > 0$ there is a matrix factor

$$\begin{pmatrix} \cos(\psi) & \sin(\psi) \\ -\sin(\psi) & \cos(\psi) \end{pmatrix} \text{ or } \begin{pmatrix} \cosh(\psi) & \sinh(\psi) \\ \sinh(\psi) & \cosh(\psi) \end{pmatrix} \quad (3)$$

where $\psi \equiv (\pi/\omega_e)(2\alpha_0)^{1/2}$. The axial (z) motion is stable if the two eigenvalues of the product of the matrices in equation (3) are complex. Thus, we have stability if $[\cosh(\psi) \cos(\psi)]^2 < 1$, or $\psi < 1.875$ or

$$\alpha_0 < 0.178 \omega_e^2 \quad (4)$$

This inequality implies stability in the transverse directions; that is, the rate of switching between the mirror and anti-mirror configurations must be rapid compared with the 'natural' frequency in the mirror field $(\alpha_0)^{1/2}$.

Equations (2) with $\alpha(t) = \alpha_0 \sin(\omega_e t)$ can be solved approximately in the limit where $\alpha_0 \ll \omega_e^2$ (ref. 24). Let $x = X + \xi$, where $X(t)$ is slowly varying on the timescale $1/\omega_e$, and $\xi(t)$ is rapidly varying. Notice that $X(t)$ is analogous to the guiding centre position of a charged particle in a magnetic field while ξ represents the rapid gyration. Then, $d^2\xi_x/dt^2 = -\alpha_0 \sin(\omega_e t) X_x$ or $\xi_x \approx (\alpha_0/\omega_e^2) \sin(\omega_e t) X_x$ and $d^2X_x/dt^2 \approx -\langle \alpha \xi_x \rangle \approx -(1/2)(\alpha_0/\omega_e^2) X_x$, where the angular brackets denote a time average over the interval $2\pi/\omega_e$. Similar formulae describe the y and z motion. Hence, the angular frequency for the slow motion is $\Omega_x = \alpha_0/(\omega_e\sqrt{2})$, $\Omega_y = \alpha_0/(\omega_e\sqrt{2})$, or $\Omega_z = \alpha_0\sqrt{2}/\omega_e$, for the x, y or z motion. For the guiding centre motion,

$$d^2X_j/dt^2 = -\partial V_e/\partial X_j$$

where

$$V_e(X) = \frac{1}{4} \left(\frac{\alpha_0}{\omega_e} \right)^2 (X_x^2 + X_y^2 + 4X_z^2) \quad (5)$$

is an effective potential, and $j = x, y$ or z . Note that the rapid motion ξ is out of phase with the mirror field $b(t)$: during the intervals in which the mirror field is attractive, $\alpha(t) > 0$ for, say, the x motion, the x displacement is $|X_x| + |\xi_x|$, which is larger than its value, $|X_x| - |\xi_x|$, during the intervals where $\alpha(t) < 0$. Hence, the attractive force exceeds the repulsive force. Note also that although $|\xi_j/X_j| \approx \alpha_0/\omega_e^2 \ll 1$, $|d\xi_j/dt|$ is not in general small compared with $|dX_j/dt|$.

Equations (2) and (5) can readily be generalized to account for the full spatial dependence²⁵ of the magnetic field $b_z(r, z)$ of one (or more) coaxial wire loops for $b_0^2 \ll B_0^2$. In place of equation (2), we write

$$\frac{d^2x_j}{dt^2} = \alpha(t) F_j(x) \quad (6)$$

for $j = x, y$ or z . Here, $\alpha(t) = \alpha_0 \sin(\omega_e t)$ with α_0 defined as before, below equation (2), and $F_j \equiv R^2 \nabla_j \{2b_z(r, z, t)/[3b_0(t)]\}$. An approximate solution is again obtained by letting $x = X + \xi$, where $d^2\xi_j/dt^2 = \alpha(t) F_j(X)$ or $\xi_j \approx -(\alpha/\omega_e^2) F_j(X)$. The slow, guiding centre motion obeys the equation

$$\frac{d^2X_j}{dt^2} = \sum_i [\partial F_j(X)/\partial X_i] \langle \alpha \xi_i \rangle = -\partial V_e/\partial X_j \quad (7)$$

where

$$V_e(X) = (1/4) (\alpha_0/\omega_e)^2 [F(X)]^2 \quad (8)$$

is the effective potential.

Figure 1 shows a contour plot of $V_e(r, z)$ obtained from equation (8) and the actual field $b_z(r, z)$ of a single circular coil of radius R . The lowest values of the effective potential are seen to occur along the z -axis, where the effective potential is

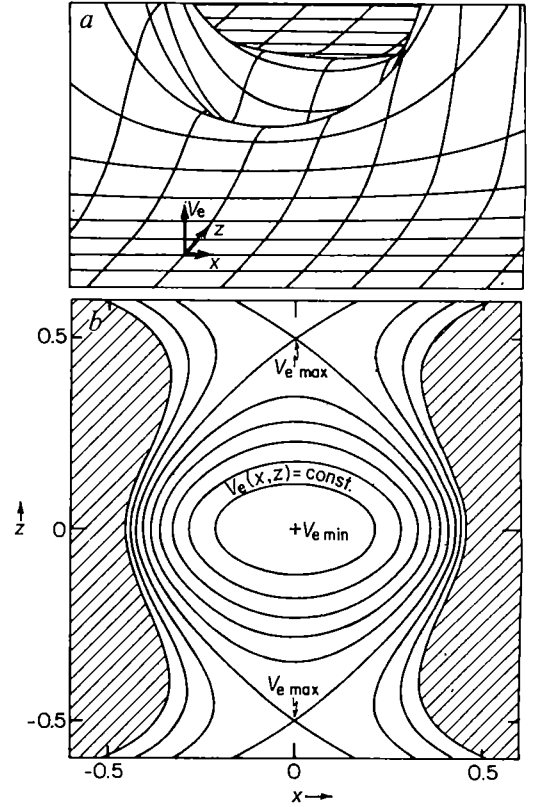


Fig. 1 Representations of the effective potential from equation (8) for the case where the b -field is caused by a single circular wire loop of unit radius in the x - y plane. *a*, An isometric view of the potential; *b*, the equipotentials. The two points denoted by V_m are separatrices. The trapped particles occupy the region $V_e \leq V_{\max} = 0.082 (\alpha_0/\omega_e)^2 R^2$. The equipotential contours are uniformly spaced with $\Delta V_e = V_{\max}/6$.

$V_e(0, z) = (\alpha_0/\omega_e)^2 z^2 [1 + (z/R)^2]^{-5}$. Thus, the 'top' of the effective potential, the separatrices of V_e , occur at $z = \pm R/2$ where $\max(V_e) \approx 0.0819 (\alpha_0 R/\omega_e)^2$.

Influence of collisions

Here, we consider the 'equilibrium' of a magnetically confined classical collisional gas in an oscillating mirror/anti-mirror field [$\alpha = \alpha_0 \sin(\omega_e t)$ in equation (6)]. Of course, the equilibrium will not be a simple Maxwell-Boltzmann distribution. For the present purposes the equilibrium can be described by the single particle distribution function (or phase space density) $f(x, v, t)$ which is a function of position x and velocity v , and which satisfies the Boltzmann equation

$$Df/Dt \equiv \partial f/\partial t + (v \cdot \nabla) f + (a \cdot \nabla_v) f = C(f, f) \quad (9)$$

where $C(f, f)$ is the Boltzmann collision integral, and $a_j = \alpha(t) F_j(x)$. A dimensionless measure of the 'strength' of the scattering is R/λ_0 . Here, $\lambda_0 \equiv [n(0)\sigma_{\text{tot}}]^{-1}$ is the mean-free path at the centre of the trap [at which point the number-density is $n(0)$], σ_{tot} is the total scattering cross-section, and R is the radius of the current loop (which is larger than the radius of the trapped gas). For the case of spin-polarized atomic hydrogen the scattering at low temperatures is predominantly the isotropic (S-wave) scattering of identical ('b' state) particles so that $\sigma_{\text{tot}} = 4\pi a_s^2$, where $a_s \approx 0.72 \times 10^{-8}$ cm (ref. 26). Thus, $\lambda_0 \approx 1.6$ cm [10^{15} cm⁻³/ $n(0)$].

Approximate solutions to equation (9) can be developed using the smallness of two parameters: $\varepsilon \equiv \alpha_0/\omega_e^2 \ll 1$ and $\delta \equiv \varepsilon R/\lambda_0 < 1$. We let $f = f_0 + f_1 + f_2 \dots$, where the zeroth approximation is

$$f_0(x, v, t) = K \exp(-H_0/T) \quad (10)$$

with

$$H_0(x, v, t) \equiv \frac{1}{2} [v - u(x, t)]^2 + V_e(x)$$

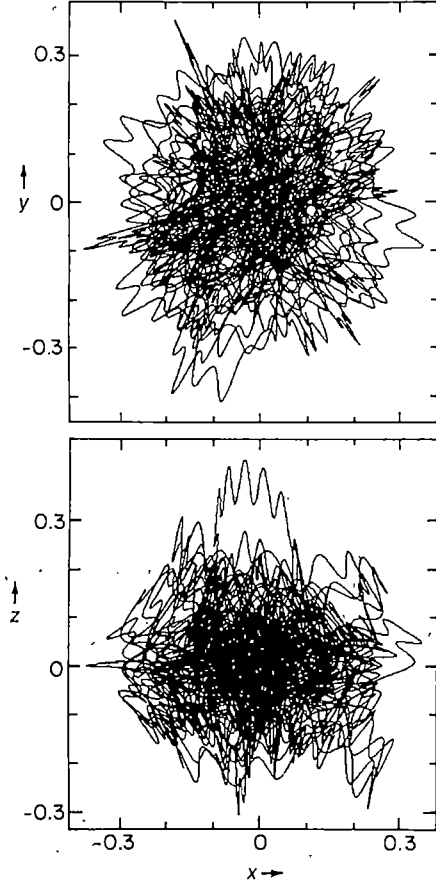


Fig. 2 Projections of the trajectory of a test particle for the conditions where $\hat{T} = 3 \times 10^{-3}$ and $\varepsilon = 0.076$. The \mathbf{b} field is due to a single circular loop. This trajectory corresponds to an elapsed time of $10^3 t_e$, where $t_e = 2\pi/\omega_e$. During this interval 490 collisions occurred. A Monte-Carlo method is used to include collisions in the orbit integrations. During each time step (dt) of the fourth-order Runge-Kutta orbit integration, a random background particle velocity \mathbf{v}_b is generated with v_b distributed according to equation (10). Whether or not a collision occurs is decided by a comparison of $(dt)n_b(\mathbf{x})\sigma_{\text{tot}}|\mathbf{v}_b - \mathbf{v}_t|$ with a Poisson-distributed random variable. If a collision occurs, the test particle velocity (\mathbf{v}_t) is modified by a rotation of $\mathbf{v}_b - \mathbf{v}_t$ in the centre of the mass frame to a randomly chosen direction. The code has been extensively tested by calculation of orbits in a time-independent potential with scattering off the corresponding Maxwell-Boltzmann background distribution $f_b(\mathbf{x}, \mathbf{v})$. The time-averaged position of the particle in phase space has been found to agree accurately with $f_b(\mathbf{x}, \mathbf{v})$, as it should.

Here, $K = \text{constant}$; $T = k_B T_0/m$, with T_0 the usual absolute temperature; V_e is given in equation (8); and $\mathbf{u} \approx d\mathbf{g}/dt$ is a time- and space-dependent flow-velocity with \mathbf{g} the above-mentioned rapid component of the single-particle motion. Specifically, we take $u_j = -(d\alpha/dt)F_j(\mathbf{x})/\omega_e^2$. Therefore, \mathbf{u} differs from $d\mathbf{g}/dt$ by a fractional amount of order ε ; and $\nabla \times \mathbf{u} = 0$ and $\nabla \cdot \mathbf{u} = 0$ in that F_j is proportional to $\nabla_j b_z$ and $\nabla^2 b_z = 0$. Thus, $\mathbf{v} = d\mathbf{X}/dt + \mathbf{u} + O(\varepsilon)$. Consequently, $H_0 = 1/2 (d\mathbf{X}/dt)^2 + V_e(\mathbf{X}) + O(\varepsilon)$ is an approximate constant of the single-particle motion. (We do not consider here the more general case where f_0 includes a dependence on the z component of the single-particle angular momentum.)

Because the collisions conserve energy and momentum, the functional form of f_0 gives $C(f_0, f_0) = 0$. This f_0 does not satisfy the Boltzmann equation (9) identically in that $Df_0/Dt = (df_0/dH_0)G$, where $G = \mathbf{v} \cdot \nabla(H_0)$. However, the time average (over intervals $2\pi/\omega_e$) of G or Df_0/Dt is zero. In order to account for the oscillating contribution from Df_0/Dt in equation (9), we let $f = f_0 + f_1$, where $Df_1/Dt = -Df_0/Dt$. Because $G = 0(\varepsilon\omega_e)$, we have $f_1 = O(\varepsilon)f_0$ with $\langle f_1 \rangle = 0$. Of course, with $f = f_0 + f_1$, the collision term in equation (9) takes on the non-zero value $C(f_0, f_1) + C(f_1, f_0)$. In order to account for this contribu-

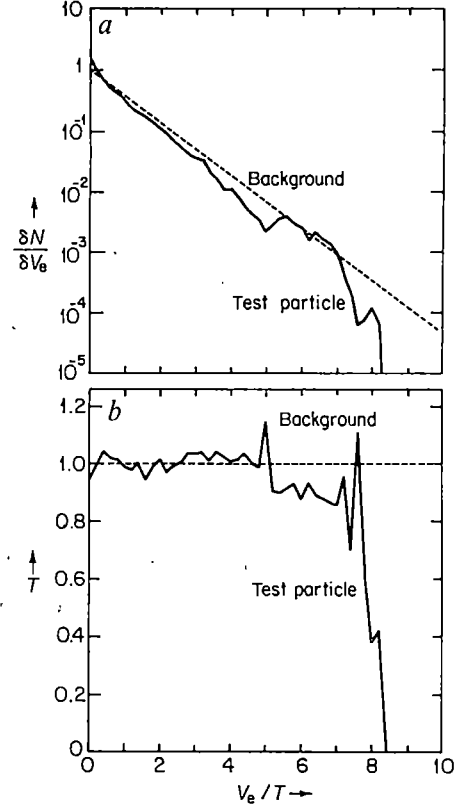


Fig. 3 *a*, The time-averaged test particle density as a function of position through its dependence on $V_e(\mathbf{x}, y, z)$. The dependence for the background is $\exp(-V_e/T)$. *b*, The dependence on V_e of the ratio of the test particle temperature ($\langle v^2 \rangle/3$) to that of the background ($T + 2V_e/3$). The test particle quantities show good approximate agreement with the theoretical background distribution function (equation (10)).

tion in equation (9), we let $f = f_0 + f_1 + f_2$, where $Df_2/Dt = C(f_0, f_1) + C(f_1, f_0)$. From this equation, we estimate $f_2 = O(\varepsilon^2|x|/\lambda_0)f_0 < O(\varepsilon\delta)f_0 \ll f_0$.

We have numerically determined the orbits of test particles in an oscillating mirror/anti-mirror magnetic field including, via a Monte-Carlo method, collisions (S-wave scattering) of the test particle from the background distribution function given by equation (10). For a discussion of the numerical results it is convenient to use dimensionless variables: $\hat{x} \equiv x/R$, where R is a characteristic length; $\hat{t} \equiv t/t_e$, where $t_e \equiv 2\pi/\omega_e$; $\varepsilon \equiv \alpha_0/\omega_e^2$; $\hat{\lambda}_0 = \lambda_0/R$, where λ_0 is the mean free path at $x = 0$; $\hat{T} = (k_B T_0/m)(t_e/R)^2$ is the dimensionless temperature; and $\hat{V}_e = (t_e/R)^2 V_e$ is the dimensionless effective potential. Figure 2 shows two projections of the test particle trajectory for an illustrative case. Notice that the axial 'width' of the orbit is approximately one-half of the transverse (x or y) width. The approximate effective potential, equation (5), implies that $\langle z^2 \rangle = (1/2)(\omega_e/\alpha_0)^2 T$ and $\langle x^2 \rangle = \langle y^2 \rangle = 2(\omega_e/\alpha_0)^2 T$. Figure 3 shows the time-averaged density and temperature as a function of position [through $V_e(\mathbf{x}, y, z)$] implied by the test particle orbit for the same case as Fig. 2.

From a large number of Monte-Carlo orbit integrations, we find that test particles are confined (for intervals $> 10^3 t_e$) only within a restricted region of the (\hat{T}, ε) plane. The oscillating field \mathbf{b} is considered to be that of a single circular coil of radius R . The confinement region of the (\hat{T}, ε) plane is bounded from above the horizontal line $\varepsilon = \alpha_0/\omega_e^2 \approx 0.19$. Also, the confinement region is bounded from below by the diagonal line $\varepsilon \approx 1.27(\hat{T})^{1/2}$. This condition arises from the fact that the size of the trapped gas increases with T (for fixed α_0 , ω_e and R) so that at a large enough value of T the gas escapes readily. In terms of dimensionless variables, the above-mentioned maximum of the effective potential is $\max(\hat{V}_e) = 3.23 \varepsilon^2$. Thus, the diagonal line corresponds to $T/\max(V_e) \approx 0.19$.

In terms of the physical variables, the conditions for confinement can be expressed as

$$I < 1,400 \text{ A-turn} \left(\frac{R}{1 \text{ cm}} \right)^3 \left(\frac{f_e}{10^3 \text{ Hz}} \right)^2 \quad (11)$$

and

$$I > 2,700 \text{ A-turn} \left(\frac{T_0}{1 \text{ mK}} \right)^{1/2} \left(\frac{R}{1 \text{ cm}} \right)^2 \left(\frac{f_e}{10^3 \text{ Hz}} \right) \quad (12)$$

where I is the number of Ampere-turns of the circular coil and $f_e = \omega_e/2\pi$. For a gas of atoms of mass M different from that of atomic hydrogen, the right-hand sides of inequalities (11) and (12) are multiplied by M/m and $(M/m)^{1/2}$, respectively. In order for equations (11) and (12) to be compatible we need

$$f_e > 1.9 \text{ kHz} \left(\frac{T_0}{1 \text{ mK}} \right)^{1/2} \left(\frac{1 \text{ cm}}{R} \right) \quad (13)$$

Some illustrative values of the parameters are shown in Table 1b.

Evaporation and viscous heating

A small but finite rate of escape or evaporation of gas atoms from the magnetic trap is expected even when inequalities (11) and (12) are satisfied. We first consider the collisional limit where $\lambda_0 \ll R$ and where an approximate treatment can be made following Jean's theory of the escape of atoms or molecules from planetary atmospheres²⁷. Clearly, from Fig. 1, the 'easiest' route of escape is along the $\pm z$ directions. We let the effective potential maximum be $V_m = \max[V_e(0, z)]$ and z_m is the axial distance to the maximum. At a distance $z_c < z_m$ where the potential is $V_c = V_e(0, z_c)$, the gas effectively becomes collisionless. Out of a Maxwellian distribution of guiding centre velocities $[f(\mathbf{X}, \dot{\mathbf{X}})]$ of equation (10) at z_c , the atoms with outwardly directed velocities greater than the escape speed $[2(V_m - V_c)]^{1/2}$ exit from the trap. The rate of evaporation from the trap can be expressed as

$$\frac{dN}{dt} \sim -(\varepsilon\omega_e)\phi N \left(\frac{V_m}{T} \right) \exp\left(-\frac{V_m}{T}\right) \quad (14)$$

Here, N is the total number of atoms in the trap, and $\phi \sim 0.07$ is dimensionless. In the collisionless limit ($\lambda_0 \gg R$), we estimate $\phi \sim 0.8 N(\sigma_{\text{tot}}/R^2)(V_m/T)$. The crossover between the two limits is at $N \sim 0.1 (R^2/\sigma_{\text{tot}})(T/V_m)$ which is $\sim 2 \times 10^{13}$ for $T/V_m = 0.15$.

The rate of energy loss of the trapped atoms due to the evaporation can be estimated using the fact that most of the escaping particles only just get over V_m . This implies a total energy loss rate of $dE/dt = 3V_m(dN/dt)$. The factor of three accounts for the fact that an escaping atom takes with it both the potential energy of its slow motion, V_m , as well as the kinetic and potential energy of its fast, 'gyratory' motion, $1/2[u(0, z_m, t)]^2 + V_e(0, z_m) = 2V_m$. Of course, the total energy of the trapped gas can be written as $E = N(K + V)$, where $\langle K \rangle$ and $\langle V \rangle$ are the average particle kinetic and potential energies, respectively. The kinetic energy is the sum of a contribution due to the slow motion (K_s) and a contribution due to the gyratory motion (K_g) and similarly for the potential energy. Because of inequality (12), most of the atoms in the trap are near the bottom of the potential well which is quadratic. Therefore, we have $\langle K_s \rangle = 3T/2$, $\langle K_g \rangle = 3T/2$, $\langle V_s \rangle = 3T/2$ and $\langle V_g \rangle = 3T/2$ so that $E = 6NT$. If evaporation were the only process affecting the total energy of the trapped gas, we would have $d(NT)/dt = (1/2)V_m(dN/dt)$ or $N[(1/2)V_m - T] = \text{constant}$. Note that our inequality (12) corresponds to $T < 0.19 V_m$.

The 'equilibrium' distribution function (10) has the non-zero flow velocity $\mathbf{u}(\mathbf{x}, t)$. Therefore, there is in general an associated viscous dissipation of the flow energy into heat in the trapped gas. This heating, denoted $(dE/dt)_v$, reflects the fact that a dynamic magnetic trap is not a closed system; the oscillating magnetic field does work on the gas. Because $\nabla \times \mathbf{u} = 0$ and

$\nabla \cdot \mathbf{u} = 0$, we have

$$\left(\frac{dE}{dt} \right)_v = 2 \int d^3x n(\mathbf{x}) \nu(\mathbf{x}) \left\langle \left(\frac{\partial u_x}{\partial x} \right)^2 + \left(\frac{\partial u_y}{\partial y} \right)^2 + \left(\frac{\partial u_z}{\partial z} \right)^2 \right\rangle \quad (15)$$

(ref. 28). Here ν is the kinematic viscosity which may be estimated as $\nu \sim \langle (\Delta \mathbf{X})^2 \rangle / \tau$, where τ is the collision time, and $\Delta \mathbf{X}$ is the displacement of the guiding centre per collision²⁹. If $\tau \ll 2\pi/\omega_e$, the displacement per collision is of the order of the mean free path λ and the usual kinetic theory formula $\nu \sim \lambda^2/\tau$ holds. However, we expect to have $\tau > 2\pi/\omega_e$ or $\tau \gg 2\pi/\omega_e$ in most of the volume of the trapped gas. In this limit the displacement per collision is of the order of the 'gyro-radius' ξ so that $\nu \sim \langle \xi^2 \rangle / \tau$. Using this estimate, equation (15) can be integrated to give

$$\left(\frac{dE}{dt} \right)_v \sim \frac{18}{(2\pi)^{1/2}} \left(\frac{T^{1/2}}{\lambda_0} \right) \varepsilon^2 NT \quad (16)$$

Here, $\lambda_0 = [n(0)\sigma_{\text{tot}}]^{-1}$, so that $\lambda_0/T^{1/2}$ is roughly the collision time at the centre of the trap. Equation (16) is in approximate agreement with the heating seen in our Monte-Carlo orbit calculations.

The combined classical influences of viscous heating and evaporative cooling are described approximately by the equations

$$\frac{dT}{dt} \sim (\varepsilon\omega_e) V_m [g\varepsilon^2(\sigma_{\text{tot}}/R^2)N - \phi(\frac{1}{2} - T')F(T')] \quad (17)$$

$$\frac{dN}{dt} \sim -(\varepsilon\omega_e)\phi NF(T') \quad (18)$$

which follow from equations (14) and (16). Here, $T' \equiv T/V_m$; $F \equiv (T')^{-1} \exp(-1/T')$ and $g \equiv 0.082(\alpha_0 R/\omega_e)^2/V_m$ with $g = 1$ for the case of a circular wire loop of radius R . If ε and ω_e are assumed to be constant, then from a high initial temperature, the trapped gas first moves comparatively rapidly towards the (T, N) curve along which $dT/dt \approx 0$. Along this curve, $|dT/dt|$ is small compared with the magnitude of the separate terms on the right-hand side of equation (17) which corresponds to an approximate balance of the viscous heating and evaporative cooling. Subsequently, the gas moves slowly down the $dT/dt \approx 0$ curve with the number of gas atoms decreasing gradually. The curve $a-c$ in Fig. 4 shows a typical cooling track. The viscous heating prevents the trapped gas from freely evolving into the region where Bose-Einstein condensation is expected to occur.

A possible strategy for reaching the Bose-Einstein region in Fig. 4 is as follows. We first alternate between cooling and filling the trap. Assume that there are initially a small number of atoms, N_i , in the trap at a 'high' temperature, T_i , so that the viscous dissipation is negligible. The starting conditions may be considered to be the point denoted by a in Fig. 4. After a short interval, the evaporative cooling brings the gas to point b , where the temperature is $\beta T_i < T_i$ and the density is $N = \gamma N_i$, where $\gamma \approx (1 - \tau_i)/(1 - \beta\tau_i) < 1$, with $\tau_i \equiv 2T_i/(V_m)$. The fact that the temperature is reduced facilitates the addition of gas to the trap. This addition may be accomplished by rapidly injecting a tight clump of atoms while the effective potential barrier V_m is momentarily reduced. A sequence of cooling and filling cycles is shown in Fig. 4.

After a sufficiently large value of N is achieved (point f in Fig. 4), it becomes advantageous to do the evaporative cooling with the mirror field strength ε slowly decreasing with time, which has the effect of lowering the walls of the trap. This value of N is below the $dT/dt \approx 0$ curve so that viscous heating is negligible initially and its relative importance diminishes with time. In this case, the $dT/dt \approx 0$ curve in the (T, N) plane moves upward and leftward as T decreases. Because the spring constant of the effective potential well ε^2 is decreasing with time, we must include a term $+(T/\varepsilon)(d\varepsilon/dt)$ on the right-hand side of equation (17) to account for the decrease of T due to adiabatic expansion. (In the absence of evaporation T would be proportional to ε .) For specificity, consider programming $\varepsilon(t)$ to

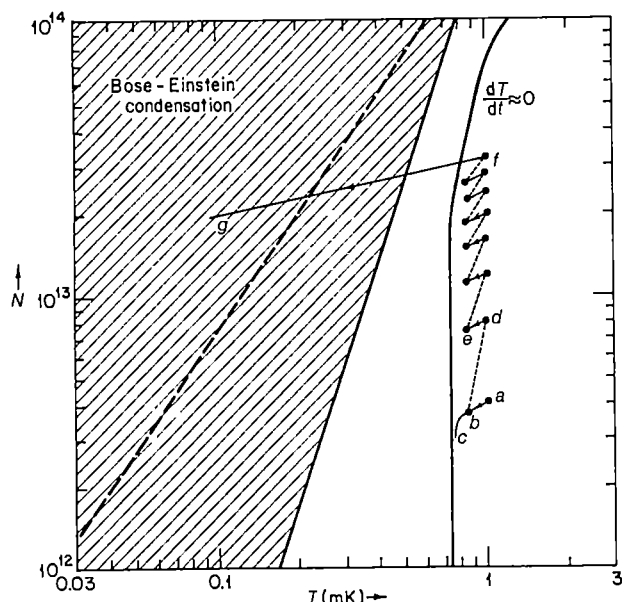


Fig. 4 Evolutionary tracks for the total number of trapped gas atoms (N) and the gas temperature (T) from equations (17) and (18) which describe the combined influences of evaporative cooling and particle loss, and of viscous heating. Except for the line f - g , the parameters are: $\epsilon = 0.06$, $T_i/V_m = 0.15$, $T_i = 1$ mK, $R = 1$ cm and $f_e = 6.9$ kHz, which are appropriate for the case where the b -field is due to a single circular loop of radius R . Along the line f - g , $\epsilon = 0.06 (T/T_i)^{1/2}$. The curve denoted $dT/dt \approx 0$ corresponds to an approximate balance of the evaporative cooling and the viscous heating. Note that equations (5) and (10) imply $n(0) \approx 1.9 (N/R^3) (V_m/T)^{3/2}$, which is $\approx 32(N/R^3)$ for $T/V_m = 0.15$. The solid straight diagonal line corresponds to $N \approx 1.7[k_B T_0/(\epsilon \hbar \omega_c)]^3$ with $\epsilon = 0.06$, which is the condition for Bose-Einstein condensation in the static potential given by equation (5). This condition for condensation can be rewritten in terms of $n(0)$ and T so that it is similar to the formula given in the introduction. The dashed straight line is the same condition for Bose-Einstein condensation but with $\epsilon = 0.06(T/T_i)^{1/2}$.

decrease in such a way that $T' = T/V_m = \text{constant}$. Because V_m is proportional to ϵ^2 we have $\epsilon = \epsilon_i (T/T_i)^{1/2}$, where i indicates the value at point f . From inequality (11), we must have $\epsilon_i < 0.19$. Equations (17) and (18) now imply

$$NT^{-\delta} = \text{constant} \quad (19)$$

where $\delta \equiv (\frac{1}{2})T'(\frac{1}{2} - T')^{-1}$. From inequality (11), we must have $T' < 0.19$. For $T' = 0.1, 0.15$ and 0.19 we have $\delta \approx 0.125, 0.21$ and 0.31 , respectively. The line f - g in Fig. 4 shows the evolutionary track. This track takes the gas into the region of Bose-Einstein condensation. Of course, in this region equations (17) and (18) must be modified to account for quantum mechanical effects²⁰, including, in particular, the fact that a non-negligible fraction of the trapped atoms may be in the condensate. Note that the timescale for the evolution from points a to g in Fig. 4 is quite rapid (~ 1 s). Once point g is reached, ϵ may be fixed. The evolution is then essentially frozen at point g .

Conclusions

Much effort will be needed to attain conditions for Bose-Einstein condensation of atomic hydrogen gas in a dynamic trap. The trapping region would need to be inside a thin non-conducting cylindrical wall with the coil windings for the b field located outside this wall in a large bath of liquid helium. For a coil of inner radius ~ 1 cm and, say, 2×10^3 turns, the inductance is ~ 20 mH and the currents needed are ≤ 10 A. The inductive impedance of the coil can be cancelled at the frequency f_e by an appropriate series capacitor. An estimation of the circuit resistance at resonance for copper wire gives a value $\sim 0.5 \Omega$. Thus, during the initial rapid evolution of the gas from points a to g in Fig. 4, the ohmic losses in the coil will be ≤ 25 W

during an interval ~ 1 s. Subsequently, at point g the ohmic losses will be a factor ≥ 10 smaller. The heat from the coil will go into boiling off a fraction of the helium from the bath.

A major problem will be that of supplying a sufficiently large number of low-temperature hydrogen atoms to fill the trap. The walls of the tube carrying the hydrogen atoms to the trap will be coated with a superfluid film of helium. Because the binding energy of a hydrogen atom to such a film is of the order of 1 K, the atoms will have a strong tendency to adhere to the walls for temperatures below 0.1 K. On the other hand, it is desirable to fill the trap with gas at a temperature below 0.01 K to avoid the need for even larger oscillating magnetic fields. However, it is not known how much below 0.1 K the hydrogen gas can be cooled by wall collisions. The accommodation coefficient is predicted to decrease monotonically with decreasing temperature³⁰, which means that the thermal disequilibrium between the wall and the gas becomes larger at lower wall temperatures when one is attempting to cool a 'hot' beam of atoms with wall collisions. An alternative approach may be to rely on hydrogen atom surface transport via diffusion along the helium film surface to the trap region (J. D. Reppy, personal communication). It may be possible to detach the hydrogen atoms from the film surface in the trap region using carefully controlled thermal³¹, sonic or magnetic gradient pulses. Another approach might involve forming a collimated and velocity-selected atomic beam which is then slowed down by a magnetic field gradient.

Another important challenge will be the detection of the Bose-Einstein condensation. Sensitive techniques are available for detecting hydrogen atoms using nuclear magnetic resonance (where 10^{14} atoms can easily be detected)³² and electron spin resonance (where 10^9 atoms can be detected)^{33,34}. In the potential well provided by the trap, the condensed hydrogen atoms (which are bosons) will tend to congregate in the low-energy states of the well (energies $\ll k_B T$), which are relatively compact in configuration space^{20,35-37}. A clear manifestation of Bose-Einstein condensation would be the appearance of a spatially compressed (condensate) component of the trapped gas. This compressed component should be evident in magnetic resonance imaging experiments. Another remarkable prediction¹⁸ is that of the appearance and precession of a spontaneous magnetization of the condensate of a hydrogen gas made up of both 'a' and 'b' states. It seems reasonable to expect that various new quantum phenomena will be found in Bose-Einstein condensed atomic hydrogen gas.

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An inherited limb deformity created by insertional mutagenesis in a transgenic mouse

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We have created an insertional mutation that leads to a severe defect in the pattern of limb formation in the developing mouse. The novel recessive mutation is phenotypically identical and non-complementary to two previously encountered limb deformity mutations, and is closely linked to a dominant mutation that gives rise to a related limb dysmorphism. The inserted element thus provides a molecular genetic link with the control of pattern formation in the mammalian embryo.

THE molecular events that guide pattern formation during embryonic development have been difficult to characterize in mammals, partly because of the difficulty in drawing a molecular connection between the phenotype of a dysmorphic mutation and the specific gene in which that mutation occurs. One means of overcoming this problem involves creating developmental mutants by insertional mutagenesis in transgenic mice and using the inserted DNA fragments as markers to clone the mutant genes. Initially, one might expect such mutants to be prohibitively rare, but, using a related approach, Jaenisch and colleagues^{1,2} have identified an embryonic lethal mutant as an insertional mutation in the $\alpha 1(I)$ collagen gene. Additional potentially interesting mutants in transgenic mice have been identified by others^{3,4}.

Recently, we created several strains of transgenic mice for experiments designed to understand the action of the oncogene *c-myc*⁵. To test for gene dosage effects, these strains were bred to homozygosity with respect to the inserted gene (in this case, a fusion construct of the mouse mammary tumour virus long terminal repeat and the mouse *c-myc* gene; MMTV-*myc*). We also systematically screened these animals for recessive lethal or dysmorphic mutations. Among seven transgenic mouse strains analysed in this way, we discovered one carrying a recessive mutation that results in a severe dysmorphism of all four limbs.

Discovery of limb deformity mutation

In preparing transgenic mice, the DNA fragments which are injected into the fertilized egg integrate into the host genome, usually at a single site, and become a stably heritable genetic element⁶⁻¹¹. From the analyses that have been done so far, no clear rules have emerged regarding the site of integration. It is, therefore, conceivable that these DNA fragments will occasionally integrate within or near, and hence disrupt the function of, a gene whose expression is essential for the normal development of the animal. For this and other reasons related to our original experimental purposes, we systematically intercrossed transgenic heterozygous animals. In this manner, the phenotype of any recessive mutation caused by the inserted MMTV-*myc*

sequences would become apparent on examination of the homozygous offspring.

In one of these lines of transgenic mice, designated the S line (previously called 165-1a)⁵, some of the offspring from heterozygous parents were abnormal. The fore- and hindlimbs of these animals were deformed, and some of the digits in their fore and hind paws appeared to be fused (Fig. 1A). We analysed the skeletons of several affected animals and noted that in all cases there was a synostosis of the long bones (radius-ulna/tibia-fibula), oligodactyly (absence of post-axial digits), fusion of some carpals, tarsals, metacarpals and metatarsals, and syndactyly (Fig. 1B). The axial skeleton appeared normal. To enhance their chances of survival, the affected animals usually had to be removed from the competition of their wild-type littermates during nursing. As adults, at least some of the affected animals have proven to be fertile.

Co-segregation

To investigate whether the mutation causing the limb deformity in these transgenic animals co-segregated with the MMTV-*myc* integration site, several mice known to be heterozygous for the limb deformity were mated either with each other or with homozygous wild-type littermates (Table 1). The latter matings

Table 1 Limb deformity phenotype segregates as a recessive trait

No. of different parents used*	Parent†									
	○×□		○×■		●×■		●×□		○×■	
	5	2	3	2	15	8	1	1	1	1
No. of litters	5		5		22		1		1	
Total no. of pups	31		26		186		10		7	
No. of pups displaying a limb deformity phenotype	0		0		39		0		0	
Per cent limb deformity phenotype	0		0		21		0		0	

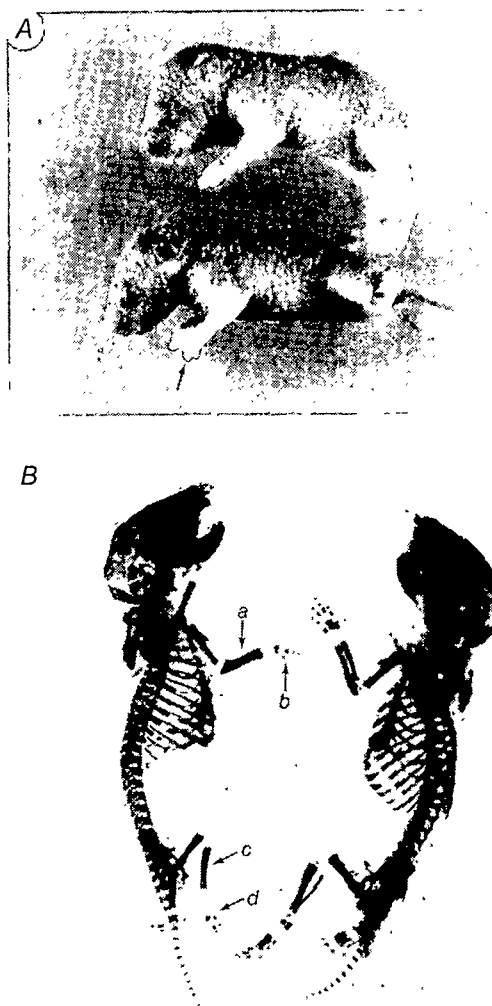
* Refers to the number of different individual wild-type, heterozygous or homozygous animals which were used to generate the numbers within each column of data.

† Circles represent females, squares males.

† Present addresses: Central Research and Development Department, Experimental Station, E. I. DuPont de Nemours & Co., Inc., Wilmington, Delaware 19898, USA (L.G.D.); Genentech, 460 Point San Bruno Boulevard, South San Francisco, California 94080, USA (T.A.S.).

Fig. 1 Characteristics of the limb deformity phenotype. The figure shows a mouse expressing the limb deformity defect compared with a normal littermate. **A**, Two 4-day-old animals from the offspring of a mating between heterozygous parents. The lower animal expressed the mutant phenotype; arrows indicate the defective fore- and hindlimbs. **B**, Skeletal structures of the homozygous mutant (on the left) and the wild-type mouse. The abnormal regions of the mutant skeleton are indicated by arrows: *a*, synostosis of the radius and ulna; *b*, oligodactyly and syndactyly of the digits of the fore paw, as well as fusion of some carpals and metacarpals; *c*, synostosis of the tibia and fibula; *d*, oligodactyly and syndactyly of the digits in the hind paw, as well as fusion of some tarsals and metatarsals.

Methods. To visualize the skeleton of the animals, the staining procedure described by McLeod²⁴ was used. Briefly, the animals were eviscerated and their pelage was removed. The samples were then placed in 95% ethanol for 7 days, followed by 24 h in acetone. The bones were stained red and the cartilage stained blue in a solution containing Alizarin red (Sigma) and Alcian blue (Sigma). The soft tissue was then cleared in a 1% KOH solution to allow the bone and cartilage to be visualized.



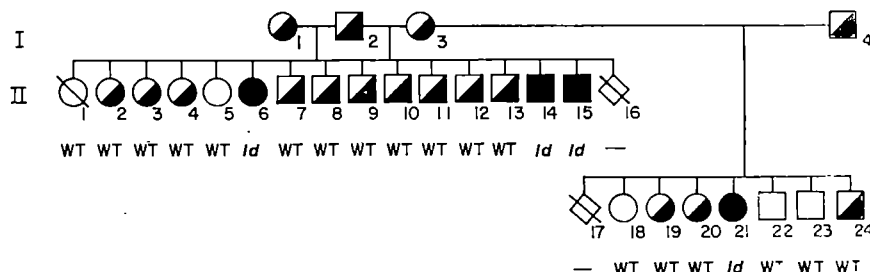
(heterozygote \times homozygous wild type) yielded no animals with the limb deformity among 57 offspring from 10 litters. Similarly, matings between homozygous affected and wild-type littermates yielded no affected offspring. The cross between heterozygous individuals, however, yielded 21% deformed animals among 186 offspring from 22 litters. These results are consistent with an autosomal recessive mode of inheritance at a single locus (39 affected individuals among 186 informative offspring: 46.5 were expected to be affected, $\chi^2_1 = 1.612$; $0.10 < P < 0.25$). To test the possibility that the limb abnormality was co-segregating with the MMTV-*myc* sequences, genomic DNAs from the tails of individuals from three representative litters were analysed to determine their genotypes (using the restriction fragment length polymorphism (RFLP) analysis described in Fig. 3 legend) (see Fig. 2). Only individuals homozygous for MMTV-*myc* sequences expressed the limb abnormality; MMTV-*myc* heterozygotes and animals entirely lacking these sequences were phenotypi-

cally normal. The recessive limb deformity thus appears to segregate with the site at which the MMTV-*myc* sequences integrated. Hereafter, *ld^{ld}* will be used to refer to this mutant locus.

Cloning of mouse genomic DNA

As the MMTV-*myc* sequences segregated with the limb abnormality, the mutation giving rise to the defect may have been caused by the integration of the foreign DNA sequences. While the disorder might be the result of expression of an inserted gene, it is more likely (see below) that the MMTV-*myc* sequences integrated into, and hence disrupted the function of, a cellular gene that participates in the development of the fore- and hindlimbs during embryogenesis. To evaluate this possibility, we cloned the mouse genomic DNA flanking both sides of the inserted MMTV-*myc* sequences in the genome of the transgenic animal.

Fig. 2 A representative pedigree showing offspring of heterozygous parent mice from the S transgenic line. The offspring (II-1 to II-24) of two different females (I-1 and I-3) and two different males (I-2 and I-4) are shown. The offspring numbered II-1 to II-16 represent a combined litter of animals from the mating of the I-1 and I-3 females with the I-2 male. All four parents were phenotypically normal; the phenotype of the individual animals is indicated by WT for wild type and *ld* for limb deformity. Males are represented by squares, and females by circles. Individuals that do not contain the MMTV-*myc* sequences are indicated by open circles or squares. Half-filled circles or squares represent transgenic heterozygotes, while completely filled circles or squares represent homozygous transgenic animals. Heterozygosity was distinguished from homozygosity using the RFLP method indicated in Fig. 3 legend. \diamond , The animal died before its sex could be determined. A slash through a symbol indicates that the animal died before its genotype could be established.



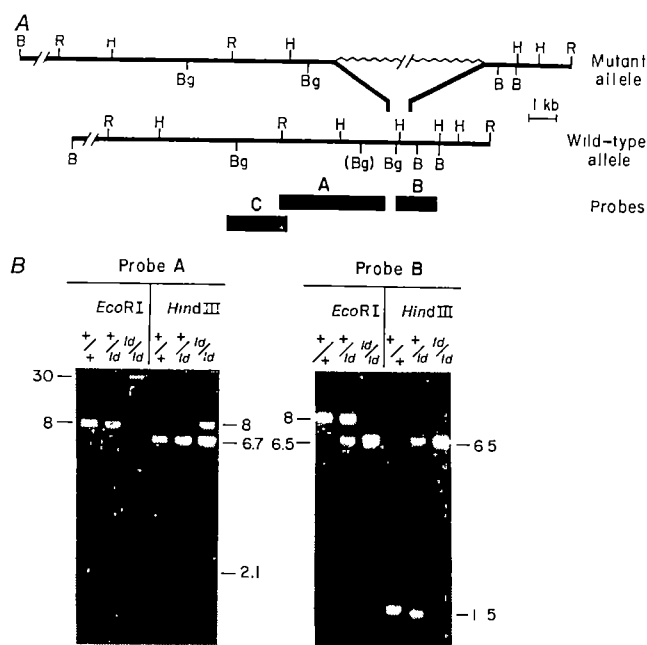


Fig. 3 Characterization of the region of the mouse genome interrupted by the inserted MMTV-*myc* sequences. **A**, Structure of a region of the mutant mouse allele containing the inserted MMTV-*myc* sequences (indicated by a wavy line) and its corresponding normal allele. The sequence between the bold lines which extends down from the mutant allele to specific sites on the normal allele is deleted in the mutant allele. Three different DNA fragments (A, B and C), represented by solid boxes, were nick-translated and used as probes in the present study. Restriction enzyme sites: H, *Hind*III; R, *Eco*RI; B, *Bam*HI; Bg, *Bgl*II. (The *Bgl*II site in parentheses is a polymorphic site and is present in only some of the alleles at this locus.) **B**, Southern blot of *Eco*RI- or *Hind*III-digested genomic DNA from an outbred CD1 mouse (+/+) or from heterozygote (*ld*/+) or homozygote (*ld*/*ld*) transgenic mice. (In this figure *ld* refers to the *ld*^{Hd} mutation.) The samples were hybridized with the nick-translated restriction fragments indicated as probes A and B in **A**. Numbers refer to the size (in kb) of the hybridizing fragments.

Methods. A portion of the mutant allele was initially cloned by preparing a genomic cosmid library with size-fractionated (30–40 kb), partial *Sau*3A-digested genomic DNA fragments from the liver of an animal of the S transgenic line. These fragments were ligated into a cosmid vector with an inactivated ampicillin resistance (*Amp*^r) gene. The cosmid vector that we used for this purpose was derived from the c2RB vector constructed by Bates and Swift²⁵ which contains both *Amp*^r and kanamycin resistance (*Kan*^r) genes. The *Amp*^r gene in the c2RB vector was inactivated by digestion at the unique *Pvu*I site in its *Amp*^r gene, filling-in the overhanging single-stranded ends with T4 DNA polymerase, and then blunt-end ligating the molecule with T4 DNA ligase. The *Kan*^r gene between the *cos* sites facilitated the growth of the modified vector in *Escherichia coli*. Cosmids which grew on ampicillin plates had acquired an *Amp*^r gene from the pBR322 sequence in the transgenic mouse DNA. (These pBR322 molecules were injected together with the MMTV-*myc* sequences in the preparation of the S line transgenic mice.) One such cosmid contained an insert comprised of a segment of MMTV-*myc* sequence together with 9 kb of mouse genomic sequence. The wild-type allele was cloned by screening a normal mouse library²⁶ which we prepared using the unmodified c2RB vector and a probe derived from the 9 kb of mouse genomic sequence that was cloned with the *Amp*^r-defective vector. Only a portion of the cloned sequences is shown in the figure.

We initially cloned 9 kilobases (kb) of mouse genomic DNA from the region flanking one side of the MMTV-*myc* sequence using a cosmid vector that was specifically modified for this experiment. This vector allowed us to select for the β -lactamase activity on the pBR322 sequences that were interspersed with the MMTV-*myc* sequences in the genomic DNA of the transgenic animal (see Fig. 3 legend for details). A region of this cloned 9-kb sequence was then used as a probe to isolate the



Fig. 4 The MMTV-*myc* integration site lies on chromosome 2. Using the Southern blot technique²⁷, we analysed wild-type BALB/c mouse and E36 hamster DNA samples, as well as DNA extracted from the 2A2B1, 2A2C2, 2A2H3 (ref. 22) and ABm5 and ABm14 (ref. 23) mouse-hamster somatic cell hybrids. A detailed chromosomal analysis of these somatic cell hybrids is given in Table 2. Genomic DNA (20 μ g) was digested with *Hind*III, electrophoresed through a 0.8% agarose gel, then transferred to nitrocellulose²⁷. A nick-translated restriction fragment, designated probe C in Fig. 3, was used as a probe for this experiment. The numbers refer to the size of the mouse and hamster hybridizing fragments. The presence or absence of mouse chromosome 2 in the individual samples of DNA is indicated by a + or -.

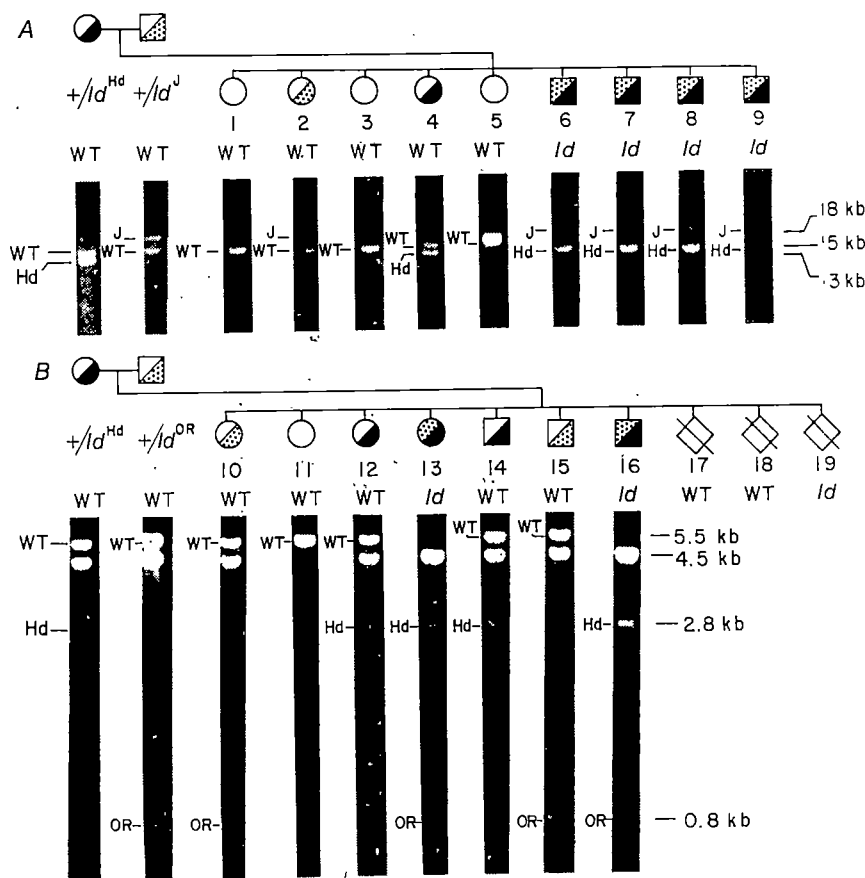
uninterrupted copy of this locus from a cosmid library constructed with DNA from a wild-type mouse. Using these clones, the structure of the locus into which the MMTV-*myc* sequences integrated was deduced (Fig. 3A).

To determine the structure of the interrupted mutant allele, genomic DNAs from a wild-type mouse (+/+) and from transgenic mice containing either one copy (*ld*^{Hd}/+) or two copies (*ld*^{Hd}/*ld*^{Hd}) of the interrupted allele were digested with *Eco*RI or *Hind*III and hybridized with sequences from either side of the insertion site (probes A and B in Fig. 3A). Both probes hybridized to the 8-kb *Eco*RI fragment that spans the insertion site in the wild-type allele (see Fig. 3B). In contrast, probe A hybridized to a 30-kb fragment and probe B hybridized to a 6.5-kb fragment in the mutant allele. The size-altered hybridizing fragments from the mutant allele probably represent the junction restriction fragments that contain a portion of the inserted MMTV-*myc* sequences. With the *Hind*III-digested DNA, probe A hybridized to 2.1-kb and 6.7-kb fragments on the wild-type sample; an 8-kb fragment was observed instead of the 2.1-kb fragment in the mutant allele. Using probe B and *Hind*III-digested DNA, a 1.5-kb hybridizing fragment (corresponding to the restriction fragment used as a probe for this analysis) was observed with the wild-type DNA; this fragment was replaced by a 6.5-kb fragment in the mutant allele. Based on these results, as well as other data including similar analyses with several other restriction enzymes (data not shown), it appears that a small region of ~1 kb has been deleted from the mouse genome as a consequence of the integration of the MMTV-*myc* sequences. Aside from this deletion, no other gross rearrangement within several kilobases of the integration site has been detected (data not shown).

Chromosomal mapping of integration site

Several morphogenetic loci that affect the development of the fore- and hindlimbs of the mouse have been identified¹². To determine whether the site of insertion of the MMTV-*myc* sequences correlates with the position of any of these loci, we chromosomally mapped the insertion segment. For this purpose, we hybridized genomic DNA from several mouse-hamster somatic cell hybrids with a probe derived from a region near the site of integration (Fig. 4). Based on the results of this

Fig. 5 Pedigree showing the 19 offspring of the mating of heterozygous parents containing the ld^{Hd} , ld^J or ld^{OR} limb deformity alleles. The phenotypes of the parents and offspring are indicated: WT, wild-type; ld , limb deformity. Symbols are as described for Fig. 2, except that \square , \circ , \otimes and \boxtimes indicate that the animal was a heterozygote. Circles or squares which are both half-solid and half-stippled refer to those animals that had inherited two defective copies of the limb deformity allele, one from each parent. **A**, Analysis of the nine offspring (1-9) from a single mating between a heterozygous S line transgenic ($ld^{Hd}/+$) mother and a Jackson ($ld^J/+$) father. The wild-type allele migrates as a 15-kb fragment, while the mutant transgenic and Jackson alleles migrate as 13- and 18-kb fragments, respectively. **B**, Analysis of the 10 offspring (10-19) from a single mating between a heterozygous S transgenic ($ld^{Hd}/+$) mother and an Oak Ridge ($ld^{OR}/+$) father. The wild-type allele migrates as a 5.5-kb fragment, while the ld^{Hd} and ld^{OR} alleles migrate as 2.8- and 0.8-kb fragments, respectively. (The 4.5-kb fragment is present on both the ld^{Hd} and ld^{OR} alleles, but not on the wild-type allele; this is due to the presence of the polymorphic *Bgl*III site (shown in parentheses in Fig. 3A) in both mutant alleles). In each case 10 μ g of genomic DNA from the parents or from their individual offspring were digested with *Bam*HI (**A**) or *Bgl*III (**B**), electrophoresed on a 0.8% agarose gel, transferred to nitrocellulose²⁷, then hybridized with a nick-translated restriction fragment (probe A in Fig. 3).



analysis, we have assigned the insertion site to chromosome 2, distal to band C1. The murine 6.7-kb hybridizing fragment was detected only in those somatic cell hybrids containing chromosome 2 or a derivative of chromosome 2 distal to band C1 (Table 2, Fig. 4). The hamster DNA yielded a 4-kb hybridizing band of moderate intensity, even under the high-stringency washing conditions used in the analysis ($0.2 \times \text{SSC}$, 0.1% SDS, 65°C); this fragment in the hamster genome must contain a region that is highly homologous with a section of the mouse restriction fragment used here as a probe.

Complementation tests

Examination of the genetic map of mouse chromosome 2 revealed the presence of several mutations which affect the development of the fore- and hindlimbs¹³. In particular, one mutation (designated ld for limb deformity) was reported to have an inheritance pattern and mutant phenotype strikingly similar to those of our transgenic mice. As we had observed with our mutant mice, in animals expressing this ld mutation the tibia and fibula were replaced by a single bone, the radius

and ulna were fused, and the bones in the fore and hind paws were reduced¹⁴⁻¹⁶. Two alleles of this mutation have thus far been observed: one at Jackson Laboratories (designated here as ld^J) and the other, possibly radiation-induced, at Oak Ridge National Laboratories (designated here as ld^{OR}). Both these mutations have been preserved by over 20 years of continuous passage.

To test for complementation between the ld^{Hd} mutation in our transgenic mice and the ld^J and ld^{OR} mutations, female transgenic heterozygotes ($ld^{Hd}/+$) were mated with heterozygous male animals from either the Jackson ($ld^J/+$) or Oak Ridge ($ld^{OR}/+$) laboratories (Fig. 5). As all three limb deformity mutations are recessive, offspring expressing the characteristic limb deformity should occur only if the mutations occur in the same complementation group. Examination of the offspring from these matings revealed several individuals expressing a limb defect that was identical to that which we observed in the transgenic mice (Fig. 5). This finding suggested that the ld^{Hd} mutation might be allelic to the ld^J and ld^{OR} mutations.

To investigate this point further, we identified RFLPs that

Table 2 Chromosomal analysis of the mouse-hamster somatic cell hybrids used in experiment of Fig. 4

Somatic cell hybrid	Mouse chromosomes present																				Reaction with mouse probe*
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	X	Y
2A2B1	+	+	+	+	-	+	+	+	+	+	-	+	-	+	+	+	+	-	-	+	+
2A2C2	+	+	+	+	-	-	+	+	+	+	-	+	+	+	+	+	+	+	+	+	-
2A2H3	+	-	+	+	-	+	+	+	+	+	-	+	+	+	+	+	+	+	+	+	-
ABm5	-	†	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
ABm14	-	+	-	-	-	+	-	-	-	-	-	-	-	-	+	-	+	-	-	-	+

Metaphase spreads prepared from somatic cell hybrids were scored for the presence of mouse chromosomes by a combination of trypsin-Giemsa and Hoechst 33258 staining. + Indicates presence of a chromosome at a frequency ≥ 0.15 copies per cell, the approximate limit of detection of the Southern blotting assay^{22,23}.

* Scored by Southern blotting, as shown in Fig. 4.

† Abm5 contains only a deletion derivative chromosome 2, $\text{del}2(\text{C1} \rightarrow \text{ter})$ ²³.

segregated with the ld^J and ld^{OR} mutations. As shown in Fig. 5A, the ld^J mutation segregated with an 18-kb *Bam*HI fragment that could be distinguished from both the 13-kb fragment that segregated with the ld^{Hd} mutation and the 15-kb wild-type fragment. In addition, the ld^{OR} mutation segregated with a 0.8-kb *Bgl*II fragment, which differs in size from the 2.8-kb and 5.5-kb *Bgl*II fragments that segregated with the ld^{Hd} and wild-type genes, respectively (Fig. 5B). (With the probe used for this experiment, the 4.5-kb *Bgl*II fragment shown in Fig. 5B is common to both the ld^{OR} and ld^{Hd} mutants.) These polymorphisms allowed us to establish the ld genotype of the offspring from the matings described above (Fig. 5A, B).

A comparison of the genotype of the individual animals with their corresponding phenotype revealed that animals that inherited two copies of the wild-type RFLP or only one copy of a mutant-linked RFLP were phenotypically normal. On the other hand, those animals that inherited a mutant-linked RFLP from both parents displayed the mutant phenotype. Therefore, the mutation reported for our transgenic animals is probably allelic to the ld^J and ld^{OR} mutations.

Development of the phenotype

There are two arguments that mitigate against a direct role for the *c-myc* gene product in the development of the ld phenotype. The more compelling argument follows from the occurrence of the two previously characterized mutations that give rise to the ld phenotype. As neither mutation involves an aberrant *c-myc* at the ld locus, it is clear that the phenotype can arise independently of an aberrantly expressed *c-myc* gene. In addition, expression of the inserted *c-myc* fusion gene occurs in the mammary gland and other tissues of heterozygotic S line transgenic mice (ref. 5 and data not shown). Assuming that there are no gene dosage effects, one would expect the mutation to be dominant if expression of the MMTV-*myc* gene is involved in the development of the ld phenotype. Indeed, the inserted fusion gene does behave as an autosomal dominant mutation with respect to the development of breast adenocarcinomas in S strain females (data not shown). Therefore, the autosomal recessive behaviour of the mutations is most easily reconciled with loss of function of the ld gene.

Role of ld gene product

One model of ld gene action holds that the normal homologue of the gene is temporally regulated during development and is expressed within the cells comprising the developing limb bud. In the mouse, the forelimbs develop first and appear as outgrowths of tissue along the side of the developing 10-day embryo¹⁷. During the next few days, the limb buds undergo extensive proximal-distal elongation, a process that may be under the control of the apical ectodermal ridge at the distal rim of the limb bud¹⁸. It has been speculated that control of

anterior-posterior pattern formation in the limb bud (the axis affected by the ld mutation) is governed by a morphogen gradient produced by the cells in a region called the zone of polarizing activity¹⁹. As the postulated gradient produced by this zone could affect the normal development of the radius, ulna and digits²⁰, the limb deformity mutation might disrupt the function of a gene normally expressed in this zone. On the other hand, expression of the gene at the ld locus could also occur in the mesenchymal cells that ultimately condense to form the bony structures within the limb. For example, the product of the ld gene could be a growth factor, a growth factor receptor or a specific cell-surface identity marker. Hopefully, additional experiments using cloned fragments from the ld locus will allow us to identify the site(s) of synthesis and action of the ld gene product.

Potential relationship to *lst* mutation

Another limb bud mutation has been mapped within two centimorgans of the ld mutation on chromosome 2 of the mouse. The mutation, Strong's luxoid (*lst*), exhibits genetic and phenotypic behaviour that is opposite from that of ld ²¹: instead of being recessive, it is dominant and instead of giving rise to too few bones in all four limbs, it gives rise to too many, that is, it is characterized by, among other things, polydactyly and duplications of the radius and tibia. As ld and *lst* were both mapped with respect to external markers and were not tested for allelism (P. Lane, personal communication), it is possible that these mutations lie much closer to one another than is suggested by the resolution of the current mapping data. We propose a very simple model that accounts for each of the mutations by allowing them to occur in the same locus. Thus, ld could represent a class of recessive mutations resulting in loss of gene function, for example by alteration of a coding sequence or promoter site, while *lst*, with its dominant behaviour, could act as a regulatory mutation in the same gene, for example by increasing the level of the ld product or allowing it to be expressed inappropriately. Fortunately, *lst* has also been preserved for the past 30 years, most recently as a frozen embryo, and will soon be available to test this possibility.

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Randomness in quantum mechanics—nature's ultimate cryptogram?

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Will a single atom irradiated by coherent light be equivalent to an infinite computer as regards its ability to generate random numbers? As described here, a search for unexpected patterns of order by cryptanalysis of the telegraph signal generated by the on/off time of the atom's fluorescence will provide new experimental tests of the fundamental principles of the quantum theory.

Dehmelt's pioneering work on the storage of single electrons and ions in macroscopic potential wells is currently being extended to neutral atoms and more complicated trapping schemes^{1,2}. These systems are interesting not only because they permit extremely accurate measurements of frequencies, or energies, but also because they are potentially useful in exploring new quantum effects. One possibility is the observation of individual quantum jumps by means of optical double-resonance methods^{1,3}. The novel aspects of this arrangement are that the fluorescent decays of a single atom can be observed, and further that the system can be reset by coherent excitation. The time delays between the resets and decays form a nominally 'random' binary telegraph signal. If the jumps are inherently indeterminate, arbitrarily long strings of random numbers can be generated by these signals⁴. In this respect quantum mechanics implies that even the simplest physical systems are equivalent to algorithms and computers of unbounded complexity⁵. This idea can be checked by searching for latent patterns of order in the sequence of fluorescent decay times. Powerful new theorems on the orbit structures of pseudo-random functions can aid in the cryptanalysis of these telegraph signals⁶. Externally induced correlations can also be exploited for precision field measurements.

The essential components of Dehmelt's shelved electron scheme for generating random fluorescence signals are shown in Fig. 1. A single atom or ion is stored in a macroscopic potential well. The present state of this technique is illustrated by the fact that a single Ba^+ ion can be localized to within $0.2\ \mu\text{m}$, or $2,000\ \text{\AA}$, in a radio frequency quadrupole trap with an effective well depth of $10\ \text{V}$; with a vacuum of 10^{-11} torr, or a residual gas density of 10^5 molecules cm^{-3} , the ion can be cooled to an equivalent temperature of $10\ \text{mK}$ and stored for hours^{1,7}. If the trapped object has an energy level structure of the type indicated in Fig. 1, a random telegraph signal can be generated by double-resonance methods³. Specifically, let us suppose that the ground state O and the excited level S are connected by a strong optical transition—that is, the matrix element $\langle O | e^{-i\mathbf{k}\cdot\mathbf{r}} | S \rangle$ is large. Then incident light (with wave vector \mathbf{k}) at frequencies matching ω_{strong} will induce transitions of an atomic electron between O and S at the Rabi flopping frequency. All the downward transitions $S \rightarrow O$ are accompanied by photon emission. A fraction of these re-radiated induced will be coherent with the incident light in virtue of emission or coherent Compton scattering. These coherent photons will be mixed with the transmitted beam. Experimentally it is much easier to detect the incoherent or fluorescent photons that are emitted transversely to the incident beam. In particular, if the strong transition $O \rightleftharpoons S$ is saturated

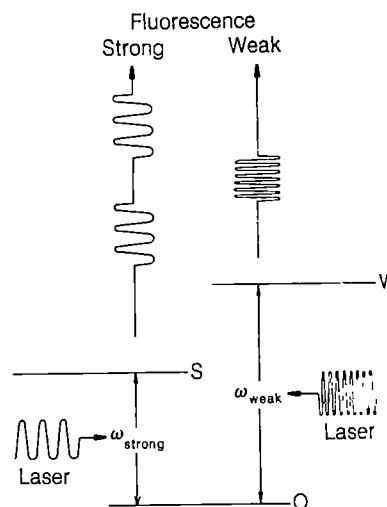


Fig. 1 Double-resonance method for generating a nominally random telegraph signal. During the time that an atomic electron is 'shelved' in state W , the fluorescence signal emanating from the $O \rightleftharpoons S$ transitions is quenched.

by the incident light, the fluorescent emission can be of the order of 10^8 photons s^{-1} , and this luminosity is sufficient to make the single trapped object visible through a microscope^{1,7}.

Suppose now, as shown in Fig. 1, that the ground state O is also weakly coupled to another excited level W . If the trapped object is simultaneously illuminated by two beams, one tuned to ω_{strong} and the other to ω_{weak} , the electron will continue to make many rapid transitions between O and S , but occasionally the incident ω_{weak} -beam will excite the upward transition $O \rightarrow W$. Clearly, while the electron is 'shelved' in state W , the fluorescence signal associated with the strong transition $S \rightarrow O$ will be shut off. However, when the electron returns to the ground state O via a spontaneous or induced decay, the strong fluorescence will switch on again. In this sense the fluorescence emanating from the strong transition is a direct indicator of the state of excitation of the weak transition³. Moreover, as the photon emission rate of the strong transition is very high, the photoelectron yield of a detector will, in first approximation, be equivalent to that generated by a steady beam of light with intensity I_0 ⁸. Similar couplings of quantum jumps to classical current pulses occur in SQUID analogue-to-digital converters⁹. The quantum jumps of the shelved electron may then be used to generate the telegraph signal shown in Fig. 2: the time intervals during which the signal is turned off (t_1, t_2 , etc.) indicate when the electron occupies state W ; the 'on' periods (T_1, T_2 , etc.) correspond to the rapid transitions $O \rightleftharpoons S$ that occur between the weak excitations of the electron from O to W .

In general, the time intervals for which the signal is off are distributed randomly with an exponential weight e^{-t/t_w} , where t_w is the decay time of the weak transition. If, as usual, A_w and B_w denote the coefficients for spontaneous and induced emission for the transition between W and O , and $U(\omega_{\text{weak}})$ is the spectral energy density of the incident ω_{weak} light, then this decay time is given by³: $t_w = [A_w + B_w U(\omega_{\text{weak}})]^{-1}$. Similarly, the time intervals for which the signal is on are distributed exponentially with weight e^{-T/T_w} , where $T_w = 2[B_w U(\omega_{\text{weak}})]^{-1}$. In principle, both of these stochastic sequences can be used to generate random numbers. For example, if the time intervals are rescaled in terms of the decay time, $\tau_i \equiv t_i/t_w$, the numerical sequence $\{e^{-\tau_i}\}$ is random and uniformly distributed in the interval $(0, 1)$. A measure of the fluctuations of the signal durations is given by the average magnitude of the difference between two arbitrarily chosen intervals, that is,

$$\langle |\tau_i - \tau_j| \rangle_{i,j} = \int_0^\infty \int_0^\infty d\tau_i d\tau_j |\tau_i - \tau_j| e^{-(\tau_i + \tau_j)} = 1 \quad (1)$$

We will shortly see that these and other statistical characteris-

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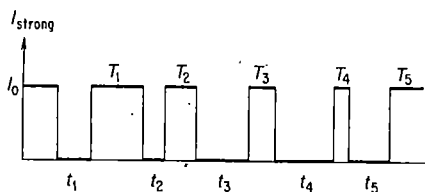


Fig. 2 Fluorescence telegraph signal. The time intervals t_1, t_2, \dots, t_i during which the signal is 'off' correspond to the 'shelving' of an electron in state W; the signal is pulsed 'on' in the intervals T_1, T_2, \dots, T_i due to the strong fluorescence transitions between S and O.

tics of random exponential sequences can be simulated by deterministic algorithms. Furthermore, previous experience with analogue and hybrid devices in random-number generation has shown that these sources have correlational defects⁴. Therefore, before any serious attempts can be made to adapt the fluorescence telegraphs for the generation of random signals, it will be necessary to check to what extent the experimentally observed time sequences $\{t_i\}$ exhibit patterns of order. A crucial advantage of using single trapped objects with simple level structures as light sources is that various types of photon correlations, such as the Brown-Twiss effect^{10,11}, Fresnel biases in superfluorescent fluctuations¹² and cascade effects¹³, are suppressed. In addition, if the fluorescence is sufficiently intense, the photoelectron fluctuations in the detectors can be treated classically; in these circumstances the instrumental jitter in the length of the telegraph pulses $\{t_i\}$ should be negligible⁸. However, random drifts in the detector gains, reference voltages and laser intensities, and interactions between the stored objects and the trapping and cooling fields, are unavoidable. Experiments are needed to determine whether the net effect of these interactions is to introduce additional elements of order or disorder.

In either case the search for correlations can be facilitated by starting with situations in which the fluorescence telegraph signals contain well-defined messages. An experimental precedent is the modulation of radioactive decay by pressure variations. One instance is the decay of the isomer ^{99m}Tc : this transition is inhibited by large changes of angular momentum and an energy release of only 2 keV. Accordingly, the decay proceeds mainly by internal conversion and the rate is very sensitive to the overlap of the initial and final electronic wave functions. This overlap can be altered by pressure variations, so that, for example, the $\sim 2.16 \times 10^4$ s half-life of ^{99m}Tc can be shortened by ~ 4 s if the ambient pressure is increased to ~ 0.1 Mbar¹⁴. Clearly, if the pressure were regularly cycled on a timescale shorter than 6 h, the existence of such periodic variations could be inferred from a stochastic analysis of the decay sequences. Similarly, the fluorescence signals from stored ions can be modulated by varying the intensities of the double-resonance lasers or by cyclically altering the trapping fields. As experiments based on the shelved electron scheme have already been carried out with 1–3 trapped $^{24}\text{Mg}^+$ ions stored within optical coherence lengths, additional information on correlations and field gradients could be extracted from the telegraph signals^{1,11,15}.

At the core of all these technical variations there remains the basic quantum-mechanical prediction that the decay time of the W-state is a random variable with an exponential distribution; this can be demonstrated rigorously using only the very weak assumptions¹⁶. In the specific case of the fluorescence telegraph this exponential behaviour is not affected by quantum measurement problems such as the 'watched-pot' or Turing paradox and observational asymmetries between prediction and retrodiction^{17,18}. The only possible source of intrinsic correlations in double-resonance measurements is a long-term quasi-periodicity that might affect the population of the S-state: however, independent experimental evidence on this point is still lacking¹⁹.

The fact that single atoms with minimal interactions with the environment can, in principle, generate arbitrarily long strings of random numbers is another remarkable aspect of quantum

mechanics. According to algorithmic complexity theory it is precisely the generation of random numbers that requires the maximum amount of complexity. In particular, n bits of information are required to specify the construction of n random binary numbers^{5,20}. The inherent indeterminacy of quantum jumps therefore not only constrains the ultimate precision of measurements but also implies that even the simplest physical systems are equivalent to arbitrarily large reservoirs of information.

The search for order in the fluorescence telegraph signals also has a bearing on the role of random variables in quantum mechanics. In the standard axiomatic formulation of probability theory, a random variable is defined as a measurable function on a probability space—where the probability space is essentially a set endowed with a normalized measure. The axiomatic development is deliberately silent concerning any requirements that the measurable functions be non-determinate or that the elements of the probability space correspond to inherently unpredictable or erratic events. Indeed, the axiomatic structure has "... applications in fields of science that have no relation to the concepts of random event and of probability in the precise meaning of these words"²¹. This basic lack of distinction between random and pseudo-random processes has several practical consequences for the cryptanalysis of the telegraph signals: (1) Inherently random events can sometimes emulate ordered patterns: for example, there is a small but positive probability that any correlation or apparent 'message' in the telegraph signals may actually be an artefact of chance events; this is the essence of the 'monkeys typing Hamlet' situation²². (2) A more serious concern is that deterministic processes can mimic random events. There is an extensive hierarchy of deterministic functions—including mixing transformations and K-systems—that can simulate the stochastic behaviour of random processes²³. These functions have recently come to popular notice in connection with studies of 'chaos'. Furthermore, the probability distributions implied by the Schrödinger wave functions of many systems are the same as the distributions which are associated with certain mixing transformations²⁴.

The nominally random decay sequences $\{e^{-\tau}\}$ of the fluorescence telegraph can also be simulated by the iteration of mixing transformations. For example, the functions

$$f(\tau) = -\ln(|2e^{-\tau} - 1|) \quad (2a)$$

$$g(\tau) = -\ln(|3e^{-\tau} - 1| - 1) \quad (2b)$$

where $\tau > 0$, are the beginning of a countably infinite sequence of mixing transformations, each of which simulates an exponentially distributed random variable. This means that if we begin with any arbitrarily chosen value of τ , say $\tau = 3.15$, and compute the successive iterates, that is, $f(3.15) \approx 0.0896$, $f[f(3.15)] = f^2(3.15) \approx 0.1880$ and so on, then term-by-term the sequence $\{f^i(3.15)\}$ appears to vary erratically, but a histogram would show that the values are in fact distributed with exponential weight $e^{-\tau}$. This behaviour is illustrated in Table 1. The fluctuations of the deterministic sequences $\{f^i(\tau)\}$ and $\{g^i(\tau)\}$ also satisfy the statistical criterion given by equation (1):

$$\langle |f^i(\tau) - f^j(\tau)|_{i,j} \rangle = 1 \approx \langle |g^i(\tau) - g^j(\tau)|_{i,j} \rangle \quad (3)$$

A random sequence would still pass this test even if only successive values were chosen

$$\langle |\tau_{i+1} - \tau_i|_i \rangle = 1 \quad (4a)$$

but the deterministic sequences have correlations that yield slightly different averages ($0 \leq i \leq 10^5$):

$$\langle |f^{i+1}(3.15) - f^i(3.15)|_i \rangle \approx 1.09; \langle |g^{i+1}(3.15) - g^i(3.15)|_i \rangle \approx 0.92 \quad (4b)$$

Needless to say, more complicated simulations require more powerful cryptanalytical attacks to detect the underlying patterns of order.

Finally we consider the highly speculative question of whether there might be a physical basis for interpreting the randomness of quantum-mechanical variables in terms of the mixing gener-

Table 1 Simulation of exponential decay by a mixing transformation [equation (2b)]

τ	$g(\tau)$	$g^2(\tau)$	$g^3(\tau)$	$g^4(\tau)$	$g^5(\tau)$...
3.15	2.0514	0.9528	0.1708	0.6368	0.8841	...
τ	0.05	0.15	0.25	0.35	0.45	...
$e^{-\tau}$	0.951	0.861	0.779	0.705	0.638	...
$\{g^i(3.15)\}^*$	0.959	0.862	0.780	0.698	0.637	...

* Histogram derived from 10^5 iterations; bin size $\Delta\tau = 0.1$; $0 \leq i \leq 10^5$.

ated by the deterministic iteration of appropriate functions. In the specific case of the fluorescence telegraph, there are two suggestive possibilities: (1) Suppose first we assume that the trapped ions that generate these signals have access to only a finite number of physical states. In particular, if we identify these states with cells in phase space—each of size \hbar^3 —it is possible to estimate their number N . For example, if a trapped ion is localized in a sphere of radius 2,000 Å, and the electron transitions involve momenta bounded by 0.5 MeV c^{-1} , it is easy to check that the maximum number of accessible phase-space cells is $N \sim 10^{17}$. (2) Suppose further that the stored ion is sufficiently isolated from its environment so that it has access to only a finite amount of information²⁵. Physically this means that the net effect of the ion's interaction with the trapping fields, laser illumination and detectors is to induce a pseudo-random walk among the 10^{17} available phase-space cells. Under these conditions the telegraph sequences $\{e^{-\tau_i}\}$ will be deterministic simulations of a discrete random variable uniformly distributed in the interval (0, 1). It can then be shown that after roughly $i \sim \sqrt{\frac{1}{2}\pi N} \sim 4 \times 10^8$ electron shelvings—or cycles of decay and re-set—each of the sequences $\{e^{-\tau_i}\}$ will merge into a terminal loop with about $\sqrt{\frac{1}{2}\pi N}$ elements. Moreover, no matter which of the 10^{17} possible states happens to initiate a telegraph signal, there is an $80 \pm 1\%$ probability that the corresponding pseudo-random sequences $\{e^{-\tau_i}\}$ will merge into the same terminal loop⁶. The practical implication of these results is that if sufficiently long strings of signals are analysed, the existence of 'messages' or terminal loops will eventually be detected. Such messages are not contained in the usual formulation of quantum theory. Since the basic cryptanalytical signature of this type of deterministic behaviour is the repetition of patterns, the relative precision of the individual observations—such as the duration of the telegraph pulses t_i and T_i —does not of course have to be comparable to one part in 10^{17} or 10^8 !

We thank P. Hammerling for helpful discussions and G. Hockney for undertaking the computer simulations.

Note added in proof: A double resonance experiment with stored $^{198}\text{Hg}^+$ ions is currently being assembled at National Bureau of Standards (Boulder, Colorado). Its characteristics are: (1) lifetime of strong transition $\sim 10^{-9}$ s; (2) Rabi flopping frequency $\geq 10^7$ Hz; (3) photon collection efficiency $\sim 10^{-3}$; (4) number of photons detected per telegraph pulse $\sim 10^3$. Since this experiment is intended for precise frequency measurement the lifetime of the weak transition is ~ 0.1 s! A faster weak transition would facilitate the search for patterns (D. Wineland, private communication).

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Dual emission-line regions in the Seyfert galaxy NGC 5929

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Several theoretical and empirical studies suggest that multiple distinct emission regions exist in the nuclei of some active galaxies. Detailed study^{1,2} of nearby objects such as M51 shows that the most important radiation of nonstellar origin can originate hundreds of parsecs from the nucleus (as defined by the stellar potential well), while binary compact objects are expected in some cluster-collapse schemes³ or in interaction-fuelled models for active nuclei^{4,5}. Here I show that the type 2 Seyfert nucleus of NGC 5929 contains two emission regions situated symmetrically about the centre of the galaxy. Slit spectroscopy shows them to be spatially as well as kinematically distinct, each with a linewidth of $\sim 200 \text{ km s}^{-1}$ and ionization levels normally associated with Seyfert nuclei. Their location in the galaxy's rotation curve suggests that they move with the galaxy's disk gas; and they probably result from energy transport from a central object of low photon luminosity but high energy output. This system furnishes a very clear example of the presence of phenomena related to the presence of a central engine, but located at a significant distance from the ultimate source of energy. The existence of such objects suggests that the usual radiatively powered, nearly symmetric models for active nuclei may be misleading in many objects.

Previous data on the Sab galaxy NGC 5929 indicated that two nuclei, or discrete emission regions, might be present. It was first identified as a type 2 Seyfert by the CfA group⁶. Intermediate-resolution aperture spectra (ref. 7 and J. Stauffer, personal communication) showed the emission-line profiles to be double-peaked. A Very Large Array (VLA) map at 6 cm by Ulvestad and Wilson⁸ showed emission dominated by two unresolved sources 1.3 arc s apart in position angle 60° , and some evidence for a weak source between them.

To confirm an association between the spatially distinct radio sources and kinematically distinct emission-line regions, a high-resolution spectrum has been obtained along the line defined by the radio sources. A Texas Instruments 800 × 800 charge-coupled device (CCD) was used with the Ritchey-Chrétien spectrograph on the 4-m Mayall telescope, covering the wavelength range 6,300–6,750 Å at a resolution (FWHM) of 2.0 Å , set by the slit width of 1.0 arc s . A 1-h exposure was obtained, resulting in peak levels of 700 counts (3,000 detected photons) per pixel (0.64 Å by 0.31 arc s) in the emission lines. The spectrum clearly shows two emission regions near the nucleus, kinematically and spatially separate (Fig. 1). The [O I] $\lambda 6,300$, 6,363 lines appear in both; this fact, the similar velocity widths ($\sim 200 \text{ km s}^{-1}$ FWHM), and high [O III]/H β in both components as found from previous data⁷, indicate that each of these regions has properties normally associated with active nuclei. The ionization levels are clearly most like type 2 Seyferts or narrow-line radio galaxies, despite the rather small linewidths. In this case, straightforward classification as a type 2 Seyfert may have obscured recognition of the unusual character of the

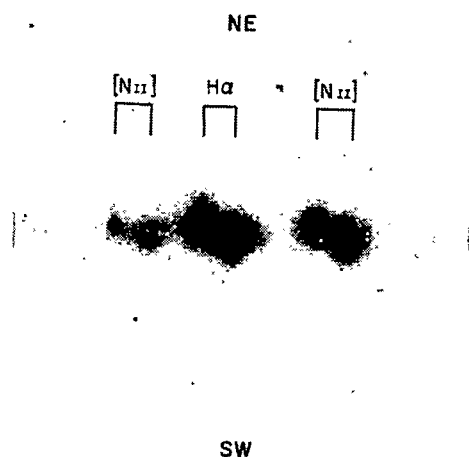


Fig. 1 The spectrum of NGC5929 in the region of $H\alpha$ and $[N II]$ $\lambda\lambda 6,548, 6,583$. The two emission regions are separated by 291 km s^{-1} and 1.3 arc s ; most of the spread along the slit is internal to the spectrograph camera. Additional weak emission between the two main peaks may be seen at $H\alpha$.

object, but the integrated line ratios and linewidths are certainly those associated with Seyfert nuclei.

Quantitative measures of the individual spectra have been performed by multiple-component gaussian fits to blended lines in each row of the spectrum, accounting for contamination of each spectrum by the other. The line ratios, relative strengths in $H\alpha$, and linewidths corrected for the instrumental profile are given in Table 1. Subtraction of the modelled components from the data provides some evidence of a weak, broad $H\alpha$ line at the centre of the galaxy; part of this emission may be seen between the $H\alpha$ peaks in Fig. 1. A comparison of the spectra of the two regions, obtained by taking the rows of the spectrum at the outer edges of each emission-line system to minimize scattered light from the other, is shown in Fig. 2.

Additional imaging data were obtained (in collaboration with R. Kennicutt) using the same CCD at the KPNO 2.1-m telescope. Exposures through filters with $75\text{-}\text{\AA}$ passbands centred on and off $H\alpha$ were obtained. Figure 3 shows the galaxy's morphology, its interacting companion NGC5930, and an enlarged portion of the $H\alpha + [N II]$ difference image. The emission-line structure is consistent with two unresolved centres of emission blended by seeing effects, with a separation close to that of the radio sources but a significantly different position angle ($\sim 85^\circ$). The structure is not consistent with a ring of emission, which might give similar spectroscopic results but a quite different physical picture.

From these data, several characteristics of the system are clearly established. Two dominant emission-line regions exist, each with a luminosity⁷ and ionization level sufficient for classification as a Seyfert 2 nucleus. They are separated by 1.3 arc s (projecting to 190 pc at an assumed distance of 34 Mpc derived by assuming $H_0 = 80 \text{ km s}^{-1} \text{ Mpc}^{-1}$), and have a radial-velocity difference of $291 \pm 11 \text{ km s}^{-1}$. The optical emission is nearly coincident with the pointlike radio sources. Finally, neither emission region coincides with the galaxy's dynamical centre; they fall instead along the rotation curve defined by disk $H II$ regions (easily traced in these data but not well seen in the low-contrast representation of Fig. 1), on either side of the nuclear velocity.

At least two plausible pictures are available to account for this configuration. Actual double nuclei, powered by separate compact objects, are occasionally expected when activity has been induced by a merger or cascading collapse of a stellar core to form compact objects. Gaskell⁹ has presented evidence for offset broad-line profiles in quasars, interpreted as evidence for merger-triggered activity, with the broad-line regions representing the cores of one of the original galaxies. In such a scheme,

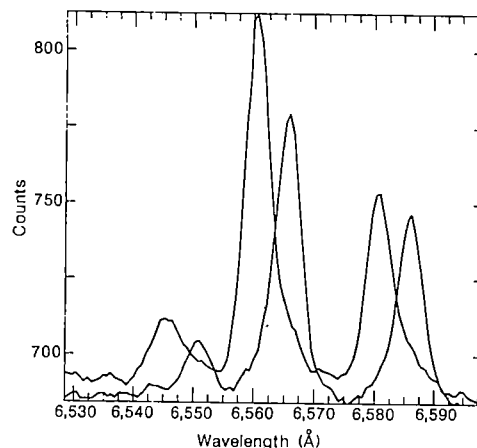


Fig. 2 Cuts through the data of Fig. 1 at the top and bottom of the emission regions, showing the spectral properties of each free from contamination by scattered light from the other. The signal-to-noise ratio is consequently lower than that obtained at the peaks, where the images of the two regions overlap along the slit due to atmospheric and instrumental spread. The wavelength scale is in the frame of the dynamical centre of NGC5929, which has an observed radial velocity of $2,516 \text{ km s}^{-1}$.

one compact object would be expected at or very close to the centre of the dominant galaxy. In most cases, a large mass ratio for the compact objects would be expected, and probably reflected in the luminosities, simply because mergers of two galaxies with nearly equal masses of the central objects are unlikely, given the large range in level of nuclear activity seen at a given galaxy luminosity.

The picture is not likely to apply to NGC5929 for several reasons. The equal spacing of the emission regions about the dynamical centre of the galaxy implies that neither represents a central object which has long existed in this galaxy. The separation of the two emission regions is too large for a binary formed by core collapse, and probably too large to represent remnant cores in a merger which has had time to produce a normal disk. The presence of a central source of radio (and perhaps broad $H\alpha$) emission would not be naturally explained. Additional circumstantial evidence against a recent merger is the fact that NGC5929 is now seen interacting with NGC5930, so that a recent merger would require that the system now be seen at a very special time.

An alternative explanation is suggested by objects showing evidence for substantial unseen energy transport between the central engine and emission regions at distances from hundreds of parsecs (as in M51^{1,2}) to kiloparsecs (radio galaxies¹⁰). The detected emission regions in these cases seem to be powered by the nucleus, but through a beamed (or 'fanned') mechanism analogous (and perhaps identical) to jets¹¹. NGC5929 may be considered within this framework as an unusually clear example of traditional active regions powered by the nucleus while lying at some distance from it. The weak central source seen at 6 cm and suspected at $H\alpha$ would then be radiation from the immediate vicinity of the central engine, while the total output of the nuclear region is dominated by twin emission regions which convert nuclear (radiation or particle) beams into line radiation (with some continuum seen at least in the radio).

The offset between radio and emission-line peaks, in this

Table 1 Emission-line systems in the nucleus of NGC5929

Line ratio	NE	SW
$[N II] \lambda 6,583/H\alpha$	0.51	0.67
$[S II] \lambda\lambda 6,717, 6,731/H\alpha$	0.59	0.81
$[O I] \lambda 6,300/H\alpha$	0.18	0.31
$[S II] \lambda 6,717/\lambda 6,731$	1.14	1.11
Relative $H\alpha$ flux	0.48	0.52
FWHM (km s^{-1})	174	192

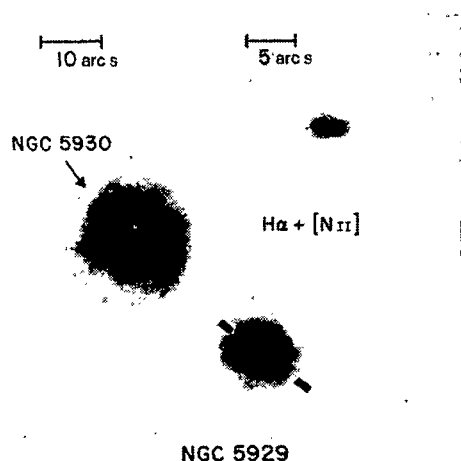


Fig. 3 NGC 5,929/5,930 as observed in a 75-Å passband including H α . The orientation and width of the spectrograph slit used to obtain the data of Figs 1 and 2 are shown by the black lines. Inset, an enlargement of the nucleus of NGC 5929 after subtraction of a continuum image, yielding a pure emission-line image. The bright portion of this image is consistent with two sources unresolved at the 1 arc s level, separated by ~ 1.2 arc s.

picture, presumably indicates that the radio peaks mark the sites of most intense ionization by the nuclear jets (or other, still collimated, forms of energy transport). The extent of optical emission is governed as much by the availability of gas as by the amount of energy available to power the emission. As there is no clear mechanism by which the directions of energy output from the nucleus should be coupled to the disk rotation 100 pc away, a 'tail' of excited material can develop as new matter is brought into the beams. The emitting clouds will become misaligned by a measurable amount with their starting points in $\sim 10^5$ yr; if the gas at $n_0 \approx 10^3 \text{ cm}^{-3}$ survives longer than this after first being ionized by the nucleus, a characteristic S-shaped configuration (like that frequently attributed to precessing jets) will be seen in the emission lines; when not resolved, this would lead to an observed centroid position offset from the points of current energy input.

If the nucleus supplies energy to the emitting region primarily in particles, they may have a role in producing the observed densities of gas through compression of the existing interstellar medium, and may also contribute to the internal velocity dispersion of the clouds. If the particle beams take the form of discrete plasmons, which would be seen directly as the 6-cm sources, the model elaborated by Pedlar *et al.*¹² to account for the densities and velocity widths in Seyfert 2 nuclei as a class might apply. In this model, the expanding plasma clouds compress the surrounding interstellar medium during thermal expansion; the medium cools after the expansion slows below $\sim 400 \text{ km s}^{-1}$ to produce the emission-line region. Some analogous process, whether discrete or continuous ejection takes place, is quite likely to be active in NGC 5929.

The properties of this nucleus suggest interesting implications for other active nuclei. Most narrow-line regions remain spatially unresolved, especially in distant, high-luminosity objects. If excitation by beams, in addition to direct photoionization from the nuclei, is important, elongated or double emission regions may be quite common, and are to be expected in some abundance with the resolution attainable with the Space Telescope. Also, note that the genuine compact object in NGC 5929, identified as the 6-cm central component and perhaps a broad H α source, is very inconspicuous, and would be difficult to detect in the absence of a surrounding gaseous disk to produce the prominent narrow-line regions. Linking statistics of active nuclei with those of central compact objects will clearly require detailed knowledge of the gaseous environments of the nuclei involved.

These data indicate that nuclear activity in galaxies can take forms significantly different from the picture developed for most

Seyfert galaxies, in which a central source of ionizing photons powers a more or less spherical ensemble of surrounding gas clouds, which then produce the emission-line spectrum. In at least some cases, the emission-line regions can be far from the energy source and dynamical centre of the galaxy, and a large part of the energy supply for these regions is asymmetrically generated by the central object. Note that if only one of the emission regions were present in NGC 5929, its peculiarity would not have been detected without considerably more detailed investigation. In at least one other galaxy, NGC 2110, the emission-line intensity and width peak at a projected distance of 220 pc from the kinematic centre¹³. In view of the results for NGC 5929, it may be argued that such objects as NGC 2110 contain 'one-sided' counterparts of the double emission-line region, and that more active nuclei may exhibit such properties when observed at high spatial resolution.

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An early-medieval account on the red colour of Sirius and its astrophysical implications

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An unresolved problem regarding ancient astronomical records is that of the star 'Red Sirius'. While Sirius today shines white with a blueish hue quite in agreement with its spectral type A1V, many Greek/Roman and Babylonian sources (although still disputed) definitely assign a red colour to this star. We now present new and apparently independent information about Red Sirius from an early-medieval manuscript. This manuscript is of Lombardic origin (8th century) and contains the otherwise lost 'De cursu stellarum ratio' by Gregory of Tours (about AD 538-593). It is preserved in the library of Bamberg¹. Red stars in ancient records are those with colour index $B - V = 1.0$ or greater. Assuming an unchanged Sirius A, this lower limit for the combined colour of Sirius A and B allows the computation of the region of pre-white dwarf Sirius B in the Hertzsprung-Russell diagram or colour-magnitude diagram (Figs 1, 2). Sirius B lies on the giant branch, which fits well with our observational and theoretical framework of stellar evolution. However, the timescale of transformation of a red giant to a white dwarf is surprisingly short.

In antiquity, Sirius was considered generally as a red star. A well-known case is Ptolemy's reference to Sirius as 'reddish' in his *Almagest*. Other classical authors, including Cicero, Horace and Seneca, concur with this opinion. Less well known is the

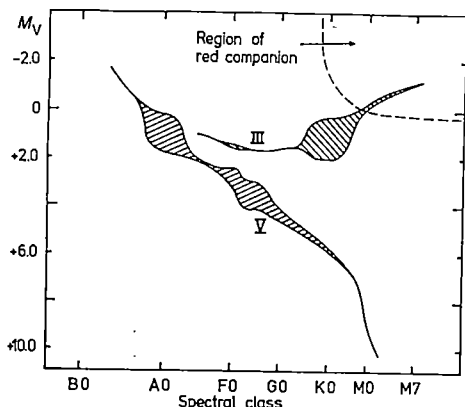


Fig. 1 Observed Hertzsprung-Russell diagram for luminosity classes III and V (after ref. 7). The variable width of each sequence indicates its varying population. The giant branch (III) runs into the region of the hypothetical red companion of Sirius A. Conversion of colour into spectral class is after ref. 9.

fact that the Romans sacrificed red-coated dogs to Sirius at the time of its heliacal rise².

The red colour of Sirius in antiquity is further substantiated by many Babylonian cuneiform texts. In his *Planetarium Babylonicum*, Gössmann³ quotes many astronomical and astrological qualities attributed to Sirius. (We follow Gössmann's translation, although not all scholars agree on the assignment of Babylonian terminology to its modern equivalent (see, for example, ref. 4).) If a colour is given, it is always red. Far-Eastern texts do not give any hint of a colour change for Sirius.

Since such a marked change of colour within historical times seems hard to accept, two alternative explanations were considered. The first one attributes the colour change to atmospheric reddening. Within 3° of elevation above horizon, Sirius indeed assumes or even exceeds the red colour of Betelgeuze. This, however, is restricted to 15 min after rising or before setting and is by no means representative of the 11-h diurnal arc of Sirius. The second argument is based on colour scintillation, which randomly changes the colour of bright stars close to the horizon. Here, simple observations of this phenomenon confirm intuitive physical insight: a white star produces as many red flashes as green or blue ones.

One should also remember that Sirius is not the brightest body in the sky. Leaving out the Sun and Moon, Sirius ranks fourth after Venus, Jupiter and Mercury, where no such controversy exists.

We have now found new information about the Red Sirius while analysing Gregory of Tours' *'De cursu stellarum ratio'*. The purpose of this work was to furnish monasteries with clear instructions on when to conduct nocturnal divine services, and for this, the rise-times of certain constellations for different months are listed. Since Gregory of Tours lived about one century before the rise of Islam, no Arabic star names appear in the manuscript, but none of the well-known classical names still in use today is used either, so this source reflects an astronomical tradition independent of classical or Babylonian skylore.

In this merovingian text, Sirius is easily identified by its rise-times and times of visibility. It is called 'stella splendida', the only case in the manuscript where this attribute of highest luminosity is given. Its proper name is 'Rubeola' or 'Robeola', meaning 'red' or 'rusty'.

This new and seemingly independent evidence for the red colour of Sirius in the past suggests that there might indeed have been an astrophysical transformation of the Sirius A/B system during the past two millennia. From an astrophysical viewpoint, the white dwarf Sirius B is the better candidate for such a change, since white dwarfs are thought to be the result of a collapsed red giant.

The total brightness of an unresolved binary system is obtained simply by adding up the intensities of each component.

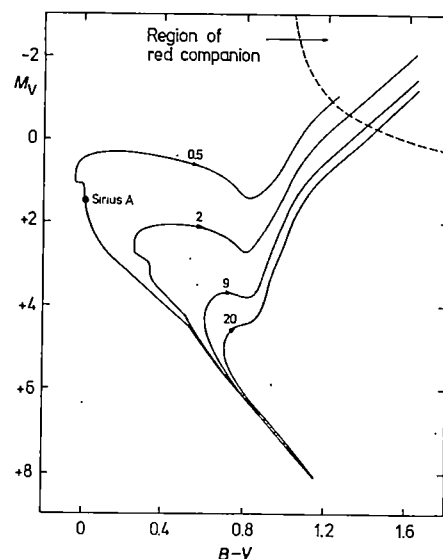


Fig. 2 Computed evolution of stars of the same age but different masses ($0.6-3 M_{\odot}$) from main sequence to the region of red giants (arrows). The direction of evolutionary tracks coincides well with predicted pre-white dwarf Sirius B colours and magnitudes. (Simplified after ref. 8 for 30% helium content and 1% heavier elements.) The numbers refer to ages in $\text{yr} \times 10^8$. Present-day Sirius A lies well on the main sequence, a further hint of its stability during the past two millennia.

Basically, the same is true for the colours. Thus, a colour-magnitude relation for Sirius B in the past may be computed by assuming an unchanged Sirius A and guessing the colour of the combined light. In antiquity, the following stars were considered to be red (Sirius excluded); Betelgeuze ($B-V=1.9$), Antares ($B-V=1.8$), Aldebaran ($B-V=1.5$), Mars ($B-V=1.4$), Arcturus ($B-V=1.2$) and Pollux ($B-V=1.0$). (All colours $B-V$ are from refs 5, 6.) Since Ptolemy's Almagest assigns no specific colour to α Centauri ($B-V=0.7$), a lower limit of $B-V=1.0$ is probably a good approximation for stars called 'red' in antiquity. Using this lower limit, a straightforward computation confines Sirius B to the region specified in Figs 1 and 2. It agrees well with the giant branch in the observed Hertzsprung-Russell diagram⁷ and the evolutionary tracks of a broad variety of stellar masses according to theoretical astrophysics⁸.

If Sirius was redder in antiquity, it must also have been brighter. Taking absolute visual magnitude, $M_V = +1.43$ for Sirius A and assuming $M_V = -1.0$ for Sirius B before change (Figs 1, 2), one obtains $M_V = -1.11$ for the combined light. With a distance modulus $m-M = -2.89$ this gives a total magnitude of -4.0 , which is about that of Venus. Like Venus, Sirius should have been visible in daylight under favourable conditions. Indeed, several Babylonian sources mention the daytime visibility of Sirius³.

Thus, Sirius B might well have changed from a red giant to the white dwarf as it appears today. However, the rapidity and smoothness of this transformation are quite unexpected, and its timescale is surprisingly short. Furthermore, no traces of catastrophic effects connected with such an event have been found. The only indication that something has happened is the somewhat higher metallicity of Sirius A, believed to have resulted from contamination by the giant's blown-off shell.

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Possible nightside source dominance in nonthermal radio emissions from Uranus

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Desch and Kaiser¹ have formulated a radiometric Bode's law from which they have attempted to estimate the low frequency, non-thermal radio power of Uranus' magnetosphere likely to be measured by the Voyager 2 spacecraft as a function of the planet's magnetic moment and the solar wind power input. They have in essence calculated what are possible necessary conditions for the generation of radio emission from Uranus. A potential problem arising from this calculation is that although their model for uranian radio emission may represent necessary conditions, they are not sufficient. I show here that, if Uranus possesses a magnetosphere, it is more likely that the radio emission is from the nightside as opposed to the dayside as assumed by Desch and Kaiser. A nightside source for the radio emissions would radically alter the predicted time for first observations of the emissions.

If a spacecraft were to approach the Earth, whose emission source is on the near-midnight high latitude nightside, from the inside its orbit, or if it were to approach Saturn, whose emission source is on the near-noon, high latitude dayside from outside its orbit, in both cases emissions would not be detected until very near encounter. Thus, although the power levels at both planets exhibit a degree of scalability, they have very different emission regions and directions that would affect any attempted predictions based on power levels alone. Earlier related work by Hill and Dessler² whose main point was to explain the ultraviolet auroral emissions using a somewhat different magnetospheric structure, qualified their predictions concerning radio emissions. In particular, they noted the uncertainty in the interpretation of the radio emissions owing to differences in beaming geometries between the planets.

One does indeed require an overall power input to a planetary magnetosphere to provide the free energy to drive planetary radio emissions. However, this energy still needs to be put in a usable form, generally field-aligned fluxes of weakly relativistic keV electrons in a region where the electron plasma frequency to electron gyrofrequency ratio, $\omega_{pe}/\omega_{ce} < 1$ (refs 3–5). More specifically, Benson⁶, using ISIS-1 satellite observations of the Earth's auroral kilometric radiation (AKR) has shown that $\omega_{pe}/\omega_{ce} < 0.2$ for the generation of extraordinary (X) mode radiation. Benson also deduced generation of weaker ordinary (O) mode radiation is allowed for $\omega_{pe}/\omega_{ce} \leq 0.9$. Since $\omega_{pe}/\omega_{ce} = 3.21 \times 10^{-3} n_E^{1/2} B^{-1}$, where n_E is the electron density in cm^{-3} and B is the magnetic field in gauss, the ω_{pe}/ω_{ce} limits of Benson yield constraints on the generation of low frequency radio emission on Uranus.

A unique aspect of Uranus is compared to Earth, Jupiter and Saturn is its near pole-on orientation towards the Sun and possible magnetic pole orientation in nearly the same direction. The possible existence of an intrinsic magnetic field has been revealed by the International Ultraviolet Explorer (IUE) spacecraft's⁷ apparent detection of uranian auroral emission that are consistent with a magnetosphere. The region of open field lines called the cusp that allows access to the ionosphere of Uranus by the shocked solar wind is thus located near the subsolar point of the planet as opposed to higher latitudes at Earth, Jupiter and Saturn. Magnetosheath plasma may thus have more direct access to the ionosphere via the cusp throat than at Earth. Given the collisional ionosphere at the base of the cusp and the cross-magnetic field transport required to move plasma outward through the cusp walls, we see that, unless a strong convective electric field is set up by solar wind/magnetosphere interaction, the cusp plasma will act as a local plasma source for the ionosphere at its base. We note that the magnitude of the cusp plasma source may be highly variable owing to solar wind dynamical evolution by entrainment and pressure waves as

discussed by Burlaga *et al.*⁸. Additionally, if these density variations are sufficiently great, the situation may become similar to the immersion of Saturn in the jovian tail⁹ and no substantial emission would be expected from Uranus in the low density solar wind troughs.

For X-mode generation we have the constraint $n_E < 3.9 \times 10^3 B^2$, and similarly for O-mode, $n_E < 7.9 \times 10^4 B^2$. Hence for $B = 0.1$ gauss (Earth-like), $n_E \geq 39 \text{ cm}^{-3}$ will suppress X-mode and $n_E \geq 790 \text{ cm}^{-3}$ will suppress O-mode also. These are very low densities for a dayside topside ionosphere with the additional cusp plasma source and unless the magnetic field is strong (≥ 1 gauss), it appears that both emission modes will be suppressed.

An additional limitation posed by high cusp electron densities is the difficulty in maintaining field-aligned electric fields needed to produce the energetic keV electron fluxes. The high densities will tend to short them out and preclude wave trapping acceleration mechanisms as have been proposed for producing field-aligned keV electron fluxes by Hasegawa¹⁰ which are needed for the Earth's AKR.

Having shown that it is plausible that a dayside source of uranian radio emission does not exist, I next consider possible nightside generation. In this case the cusp densities should be much reduced owing both to the lack of photoionization as well as the inhibition of transportation of dayside ionospheric plasma to the nightside across magnetic field lines. Thus if $B \sim 0.1$ gauss Earth-like auroral zone type densities should be possible with $\omega_{pe}/\omega_{ce} < 1$ if $n_e < 10\text{--}100 \text{ cm}^{-3}$. Thus strong emission will occur provided the keV electron fluxes can be produced in sufficient quantity. To explore this, I examine two limiting cases of the uranian magnetosphere. These two cases represent (1) the limits of high mass loading due to pickup plasma by the magnetosphere from the planet's satellites resulting in a rotationally driven, convection-dominated magnetosphere¹¹ like Jupiter and Saturn or (2) at the opposite extreme, no mass-loading and a Faraday disk dynamo magnetosphere¹². In the first case the emissions may be expected to be dominated by plasmashet related MHD instability processes and electron acceleration by wave-trapping as they apparently are at Saturn¹³. In contrast, in the low mass-loading limit, one would expect the Kelvin-Helmholtz instability operating on the magnetopause boundary to generate field-aligned surface MHD waves. Through mode conversion¹⁰, these waves will trap and similarly accelerate magnetosheath electrons into the nightside cusp/magnetosphere boundary region. Since the waves will only be driven nightward, the electrons will be accelerated only in the direction of the nightside pole. This limiting case presents some difficulties if the IUE observations are correct, since only a nightside auroral and radio emission would be expected. In contrast, in the high mass-loading limit, the centrifugally driven plasmashet interchange instabilities can drive surface waves that can accelerate electrons into both dayside and nightside cusp regions. In this case, since $\omega_{pe}/\omega_{ce} > 1$ in the dayside cusp-magnetosphere boundary region, only aurora would be generated on the dayside. However, both aurora and radio emission can be generated on the nightside where $\omega_{pe}/\omega_{ce} < 1$ is likely. Hence, the dominant radio emission from Uranus is expected from the nightside cusp-magnetosphere boundary region, which should not be observed until uranian encounter. We also note that of the dayside aurora of Uranus are genuine, mass-loading of the magnetosphere by its satellites is probably an important process within the framework presented here. If, however, planetary rotation and magnetic merging combine to give a large enough helical twist to the tail field lines, the resulting Birkeland currents that flow into the dayside polar cap may give rise to a dayside polar aurora even in the low mass-loading limit¹².

In conclusion, it is not possible to exclude either the Desch and Kaiser, or the Hill and Dessler power scaling relations. Clearly, however, their predictive power is marginal in that all of these authors have assumed a dayside radio emission source on Uranus which may, according to the arguments presented here, be either very weak or non-existent.

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Predicted chemistry of the deep atmosphere of Uranus before the Voyager 2 encounter

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The Voyager 2 spacecraft will encounter Uranus in January 1986, and will provide the first spacecraft observations of that planet. It carries an infrared interferometer spectrometer (IRIS) which has already detected a variety of infrared active gases in the atmospheres of Jupiter, Saturn and Titan¹⁻⁴. Some of the detected gases are not in chemical equilibrium but have sources in the deep atmosphere. IRIS observations of these non-equilibrium gases, in particular PH₃ (mixing ratio $X_{\text{PH}_3} \approx 6 \times 10^{-7}$) and GeH₄ ($X_{\text{GeH}_4} \approx 7 \times 10^{-10}$) on Jupiter¹ and PH₃ ($X_{\text{PH}_3} \approx 1 \times 10^{-6}$) on Saturn³, can be used to deduce the strength of convective mixing in the deep atmospheres of Jupiter and Saturn^{5,6}. Similar deductions could be made about convective mixing rates in the deep atmosphere of Uranus if models of the equilibrium chemistry and thermochemical kinetics were available to help interpret IRIS observations of the visible Uranus atmosphere. We describe here the results of comprehensive thermochemical equilibrium and chemical kinetic calculations for Uranus. We predict that the most abundant non-equilibrium trace gas derived from the deep atmosphere of Uranus is N₂; other important non-equilibrium species include HCl, HF, GeH₄, C₂H₆, PH₃, H₂Se, CH₃SH, CO, CH₃NH₂, CH₃OH, and CO₂. Some of these species are detectable potentially by the Voyager instruments.

We assume that Uranus, like Jupiter and Saturn, has a deep convective atmosphere. Use of this assumption also leads to reasonable interior structure models⁷, but this is still uncertain because of the apparent lack of a large internal heat source on Uranus⁸. The present results refer to an arbitrary but plausible compositional model in which H₂ and the noble gases (He, Ne, Ar, Kr, Xe) are present in solar abundance⁹, H₂O, NH₃, H₂S and many 'heavy' elements (atomic number ≥ 3) are enriched 100 times relative to solar abundance, and CH₄ is enriched ≈ 10 times. This model, which is based on the low-temperature equilibrium condensation sequence outlined by Lewis¹⁰ and Prinn and Fegley¹¹, is one of several that we are investigating. It is consistent with deductions from visible¹² and infrared¹³ spectroscopy and interior structure models⁷ that Uranus is enriched in 'heavy' elements relative to solar abundances.

We constructed an adiabatic temperature-pressure profile for our model Uranus atmosphere taking temperature $T = 74$ K at pressure $P = 1$ bar (ref. 13). Thermochemical equilibrium calculations were done for several hundred compounds of the elements H, He, O, C, N, S, Fe, Mg, Si, Na, Cl, K, F, P, Ge, Se, Ne, Ar, Kr, Xe, Ca, Al and Ti. The thermochemical kinetics of the important destruction reactions for selected gases were also studied. Homogeneous gas phase and heterogeneous Fe-catalysed destruction reactions for N₂ and CO were included in our model. The specific techniques used to construct our model and the thermodynamic and chemical kinetic data sources are described in detail elsewhere^{5,6,11,14-18}.

The calculated equilibrium mixing ratios of important gases as functions of temperature and pressure are illustrated in Fig. 1. The abundances of two major gases H₂O and NH₃ are appreciably reduced by the formation of dilute NH₃-H₂O solution clouds at 595 K. By the 275 K level the NH₃ abundance is less than the H₂S abundance and it is quantitatively removed from the atmosphere by precipitation of NH₄HS(s) as first suggested by Prinn and Lewis¹⁹. X_{NH_3} varies from about 10^{-9} at 200 K to 10^{-14} at 160 K, compatible with the observations of Gulkis *et al.*²⁰ that NH₃ is depleted in this region of the Uranian atmosphere. Atreya and Romani²¹ and Stevenson²² also noted that formation of NH₃-H₂O solution clouds would consume large amounts of NH₃. The excess H₂S may condense slightly higher in the atmosphere at 166 K but is probably photolysed to S₈(s) on a short timescale, while CH₄(s) condenses at 77 K. The equilibrium abundances of other gases such as HCl, HF, H₂Se, GeH₄ and P₄O₆ are reduced significantly by precipitation reactions lower in the atmosphere (see Fig. 1).

Larger enhancements of H₂O will yield solution cloud condensation at higher temperatures (and deeper levels) of the

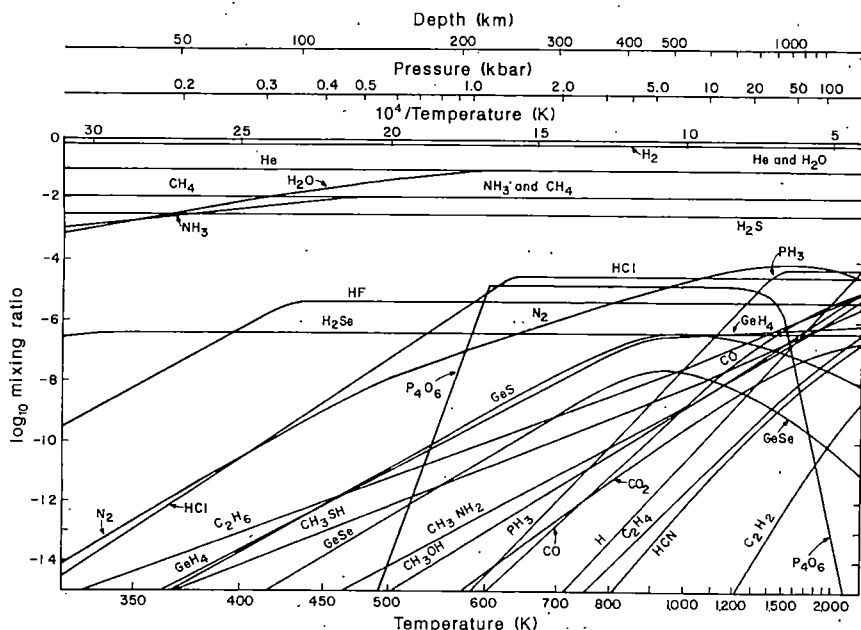


Fig. 1 Equilibrium abundances of important gases along the model adiabat in the atmosphere of Uranus. The horizontal scales indicate temperature, depth below the 300 K level and pressure along the adiabat. The constant abundances of the noble gases ($X_{\text{Ne}} = 1.5 \times 10^{-4}$, $X_{\text{Ar}} = 6.1 \times 10^{-6}$, $X_{\text{Kr}} = 2.4 \times 10^{-9}$ and $X_{\text{Xe}} = 3.4 \times 10^{-10}$) are not plotted. Precipitation of NH₄Cl(s), NH₄F(s), NH₄H₂PO₄(s), Ge(s) and GeSe(s) at 629 K, 430 K, 602 K, 868 K and 339 K, respectively, depletes HCl(g), HF(g), P₄O₆(g), Ge gases and Se gases. Solution of H₂S(g) and HF(g) in the ammonia-water clouds is a minor effect. All compounds of Fe, Mg, Si, Na, K, Ca, Al, Ti are condensed phases over the (P, T) range considered.

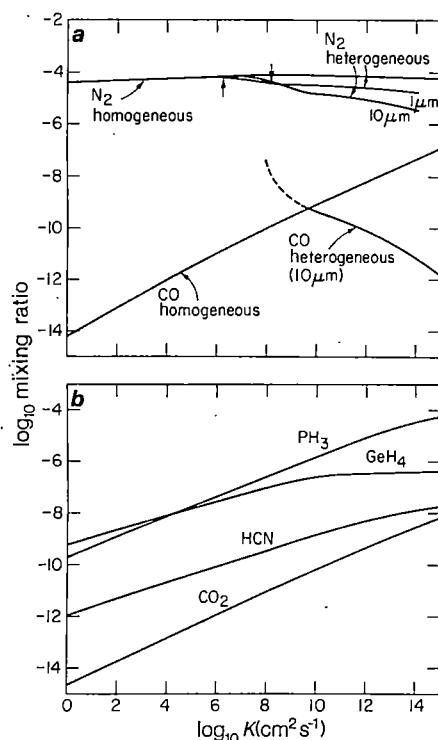


Fig. 2 *a*, Predicted N_2 and CO mixing ratios in the observable uranian atmosphere as a function of the vertical eddy diffusion coefficient K . Homogeneous gas-phase and heterogeneous iron-catalysed reactions are considered. Curves for catalysis on iron cloud particles with radii of $10\ \mu\text{m}$ (CO) and $1\ \mu\text{m}$ and $10\ \mu\text{m}$ (N_2) are shown. The dotted line on the CO heterogeneous curve indicates the predicted trend in the absence of homogeneous gas-phase reactions. Actual CO mixing ratios for $K < 5 \times 10^9\ \text{cm}^2\ \text{s}^{-1}$ will follow the homogeneous curve. *b*, As *a* but for PH_3 , GeH_4 , HCN and CO_2 . Homogeneous gas-phase reactions only are considered.

uranian atmosphere until the H_2O critical point of 647 K is reached. Because the initial clouds are very dilute NH_3 - H_2O solutions which have critical points slightly lower than that of pure water²³, the 647 K level is the upper temperature limit to a NH_3 - H_2O solution cloud base on Uranus. Latent heat effects due to solution condensation will also decrease as the cloud-base temperature approaches the critical point for the first formed (dilute NH_3 - H_2O) solution^{24,25}.

The observations of PH_3 and GeH_4 on Jupiter¹ and of PH_3 on Saturn³ at concentrations orders of magnitude greater than their equilibrium abundances in the cool visible regions of the jovian and saturnian atmospheres have been interpreted in terms of rapid convective mixing from the hot deep atmospheres where these species are more abundant^{5,6,14}. We have applied a similar model to Uranus to predict concentrations of non-equilibrium gases such as N_2 , GeH_4 , PH_3 , CO, CO_2 , and HCN in the uranian upper atmosphere as a function of the rates of the relevant homogeneous and heterogeneous destruction reactions and the (assumed) strength of convective mixing in the deep uranian atmosphere. Iron-catalysed heterogeneous $N_2(g)$ and $CO(g)$ destruction reactions were considered using the model of Prinn and Olaguer¹⁷. This treatment gives firm lower limits to the N_2 and CO mixing ratios, because Fe(liquid) condenses at 6,000 K (2.4×10^7 bar) so Fe particles in the 1,000–2,000 K region are unlikely. The results of these calculations are illustrated in Fig. 2 where the predicted mixing ratios are plotted as a function of the vertical eddy diffusion coefficient K .

No observational estimates are available for the vertical eddy diffusion coefficient K in the deep uranian atmosphere. However, various *a priori* estimates of K are possible. Assuming that free convection is a dominant mode for vertical heat transport in the deep uranian atmosphere, we can estimate K from the relationship, $K \approx H(\phi/\rho\gamma)^{1/3}$ where H is the pressure scale

Table 1 Predicted non-equilibrium trace gas abundance in the observable atmosphere of Uranus for 100 times enriched compositional model ($K = 10^7$ – $10^8\ \text{cm}^2\ \text{s}^{-1}$)

Gas	Quench temperature (K)	Predicted mixing ratio
N_2	1,205–1,390	$(5.0\text{--}6.3) \times 10^{-5}$
PH_3	1,058–1,102	$(1.0\text{--}2.3) \times 10^{-7}$
GeH_4	775–810	$(5.0\text{--}10) \times 10^{-8}$
CO	846–885	$(3.2\text{--}10) \times 10^{-11}$
HCN	1,299–1,357	$(1.6\text{--}3.5) \times 10^{-10}$
CO_2	787–832	$(3.2\text{--}10) \times 10^{-12}$

height, γ is the ratio of the heat capacity at constant pressure to the gas constant, ρ is the atmospheric density, and ϕ is the upward heat flux carried by free convection. Taking $\phi \approx 10$ – $100\ \text{erg cm}^{-2}\ \text{s}^{-1}$ from ref. 26 yields $K \approx (5\text{--}10) \times 10^7\ \text{cm}^2\ \text{s}^{-1}$ at the 1,000 K level in our atmospheric model. These values suggest more sluggish convective mixing on Uranus than on Jupiter or Saturn where $K \approx 10^8$ – $10^9\ \text{cm}^2\ \text{s}^{-1}$ in the deep atmosphere^{6,16,27–29}.

Table 1 shows the predicted abundances of N_2 , CO, PH_3 , GeH_4 , HCN and CO_2 in the observable Uranus atmosphere for $K \approx 10^7$ – $10^8\ \text{cm}^2\ \text{s}^{-1}$. Specific kinetic calculations were not done for other potential non-equilibrium gases such as C_2H_6 , CH_3SH , CH_3NH_2 and CH_3OH , but quench temperatures of $\approx 1,000$ K may not be unreasonable for these gases³⁰; the resulting abundances can then be estimated from Fig. 1. Since precipitation of $NH_4Cl(s)$ and $NH_4F(s)$ may be very difficult to quench, HCl and HF are likely to be in equilibrium; however, if the low NH_3 mixing ratios ($< 10^{-6}$) inferred by Gulkis *et al.*²⁰ hold throughout the uranian atmosphere there will not be enough NH_3 to react with the HCl and HF, so their equilibrium concentrations could be much higher than indicated in Fig. 1. Non-ideal gas behaviour may increase the pressure-dependent N_2 abundance; at our highest N_2 quench temperature (1,390 K), the pressure may be three times too high³¹ and thus the predicted N_2 abundance nine times too small, while at lower temperatures the required corrections becomes smaller.

Once the Voyager observations are in hand, the inverse process, namely deduction of K values from the observed abundances of PH_3 , GeH_4 and so on, will be possible using Fig. 2.

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Steam rapidly reduces the swelling capacity of bentonite

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Sodic bentonite is widely used for industrial and scientific purposes, especially as a sealing agent¹, because of its great ability to swell in water^{2,3}. It has been proposed for use in high-level nuclear waste repositories as an impermeable barrier surrounding the waste package, or to fill tunnels, shafts and rooms. In some repositories, such as those planned in basalt, bentonite would be expected to be subjected to temperatures possibly up to 300 °C (refs 4, 5). As the waste would be approximately 600-900 m below the water table in fractured rock, the repository is expected to fill first with steam and then with liquid water^{4,6}. Reaction of bentonite with liquid water produces a minimal loss of swelling capacity, but I show here that reaction with water vapour at 150-250 °C results in rapid irreversible loss of most of the swelling capacity. This causes very large increases in permeability of sand-bentonite mixtures⁷, thereby reducing the ability of a bentonite barrier to retard the flow of groundwater in proposed high-level nuclear waste repositories.

The thermal and hydrothermal stability of bentonite has been repeatedly questioned^{6,8-10}, but transformations generally are expected to be slow enough and the duration of high temperatures brief enough that bentonite is not necessarily disqualified for use in a repository at high temperatures^{5,9,10}. Furthermore, in some environments, transformation may be limited by availability of potassium or other elements⁹⁻¹¹. In typical

hydrothermal experiments, montmorillonite was structurally unaltered, but underwent compositional changes after reaction with basaltic groundwater for 96 days at 360 °C, 30 MPa (ref. 10), and after reaction with basalt plus groundwater for 379 days at 300 °C, 30 MPa (ref. 5). Large reductions in the ion-exchange capacity¹² and small reductions in the swelling ability^{13,14} of bentonite were observed after heating to temperatures of 100-300 °C, but irreversible dehydration, which would imply a major mineralogical change, was not observed after heating for 340 days at 370 °C (ref. 5).

On the other hand, rapid and severe reduction in the swelling ability of bentonite because of exposure to steam has recently been observed⁷. In a series of experiments the permeability of sand-bentonite mixtures (25% bentonite, 75% basalt or quartz sand, with dry bulk densities of ~1.6 g cm⁻³), as measured by flow of liquid water, increased by up to 10⁵ as a result of prior exposure to water vapour at temperatures between 250 °C and 260 °C (ref. 7). The increase in permeability was due to a large, irreversible decrease in the swelling capacity of the bentonite. Here I present data on the effects of water vapour on bentonite at temperatures between 150 °C and 250 °C.

The methods and results are shown in Table 1. The untreated bentonites swell to very high specific volumes in water. For example, Envirogel expands to a specific volume of 37 ± 4 cm³ g⁻¹, and forms a thick gel at specific volumes of 30 cm³ g⁻¹ or less. The maximum specific volume in water is defined here as the swelling capacity.

Dry heating to 250 °C for 7 days with no added water seems to reduce the swelling capacity slightly (sample 5), as observed previously^{13,14}. This reduction may have resulted from reaction with water of hydration before the sample dried, as the following experiments suggest.

Heating the bentonite to 250 °C in a closed system, with a limited amount of water, caused a great change in wetting and swelling behaviour. The unreacted material forms a sticky, gummy paste when mixed with water, but the reacted material is not cohesive in water, and has greatly reduced swelling capacity (samples 6-11, 19-21). All four bentonites were affected similarly. Bentonite initially contains about 8% water of hydration; even this amount of water, if retained during heating, is enough to severely alter the swelling properties of the bentonite

Table 1 Effects of heat and water vapour on swelling capacity of bentonite

Sample no.	Bentonite	Mass of bentonite (g)	Water added (g)	Water composition	Temperature (°C)	Total water* bentonite	Swelling capacity (cm ³ g ⁻¹)
1	Envirogel	—	—	—	Untreated	—	37
2	SWy-1	—	—	—	Untreated	—	37
3	Montmorillonite 27	—	—	—	Untreated	—	39
4	National Standard	—	—	—	Untreated	—	25
5	Envirogel	4.84	0	dw	250	0	28
						(heated dry)	
6	Envirogel	4.84	0	dw	250	0.08	9
7	Envirogel	4.84	0.10	dw	250	0.10	9
8	Envirogel	4.84	0.19	dw	250	0.12	7
9	Envirogel	4.84	0.34	dw	250	0.15	4
10	Envirogel	4.84	0.82	gw	250	0.25	5
11	Envirogel	4.84	0.82	dw	250	0.25	5
12	Purified Envirogel	1.00	0.20	dw	250	1.08	7
13	Envirogel	2.00	2.12	gw	250	1.14	10
14	Envirogel	0.25	5.00	dw	250	20†	>20†
15	Envirogel	0.25	5.00	gw	250	20†	>20†
16	Altered Envirogel	0.25	5.00	gw	250	20	4
17	Envirogel	4.84	0.34	dw	200	0.15	9
18	Envirogel	4.84	0.34	dw	150	0.15	13
19	SWy-1	4.46	0.29	dw	250	0.14	5
20	Montmorillonite 27	4.84	0.34	dw	250	0.15	13
21	National Standard	4.84	0.34	dw	250	0.15	11

Samples of bentonite were reacted with deionized water (dw) or basaltic groundwater (gw) for 5-7 days (5 days for samples 5 and 11; 7 days for the others) in small stainless steel or Inconel autoclaves. The composition of the groundwater was 3.0 mM NaCl, 2.0 mM Na₂SiO₃, 2.0 mM NaF, 1.1 mM Na₂SO₄, 0.7 mM NaHCO₃, 0.6 mM Na₂CO₃, 0.03 mM CaSO₄, adjusted to pH 9.9 with HCl. The autoclave volumes were 7.6-7.9 cm³, except for sample 20 (5.4 cm³). After reaction, the swelling capacity of the clay was determined by dispersing 0.10-0.15 g (dry weight) of clay ultrasonically with 4 ml of water in graduated conical centrifuge tubes at ~22 °C, allowing the clay to settle and measuring the expanded volume. The expanded volumes did not change after several months of standing. The error in swelling capacity is ±10%. The temperature accuracy is ±5 °C. The bentonites were from the following sources: Envirogel, Wyo-Ben, Inc.; SWy-1, Clay Minerals Society; Montmorillonite 27, Ward's Natural Science Establishment, similar to American Petroleum Institute Montmorillonite 27; National Standard, Baroid Corporation. Except for samples 12 and 16, the only pretreatment of the bentonite consisted of drying, crushing and sieving by the suppliers. Except for quartz and plagioclase, the samples were nearly pure montmorillonite, with sodium as the predominant exchangeable cation. Smaller amounts of potassium feldspar (probably sanidine), illite or mica, calcite, gypsum and apatite were also present. Most of the experiments were done with Envirogel (Wyo-Ben, Inc.), which contains ~80% montmorillonite and is described elsewhere¹⁰. Purified Envirogel (sample 12) was prepared by sedimentation in water to remove about 20% sand and silt. Altered Envirogel (sample 16) was prepared by previous reaction for 6 days at 250 °C, water/bentonite ratio = 0.15.

* Includes (8 ± 4)% initial water content of bentonite, except for sample 5, which was heated dry in an open vessel.

† Water and bentonite mixed ultrasonically before heating. Sample absorbed all water to form a gel, and remained gelled after hydrothermal treatment.

Table 2 Alteration of bentonite at controlled P_{H_2O}

Bentonite	Temperature (°C)	P_{H_2O} (MPa)	Relative humidity (%)	Swelling capacity ($\text{cm}^3 \text{g}^{-1}$)
Envirogel	250	3.7	93 ± 2	10 ± 1
Envirogel	Unaltered	—	—	37 ± 4

Bentonite was heated to 250 °C in a vessel at atmospheric pressure, the vessel was evacuated, water vapour introduced at $P_{H_2O} = 3.7$ MPa, the pressure was maintained for 18 h, and then the pressure was released, the bentonite was cooled to room temperature and the swelling capacity was determined. Liquid water was never present during alteration; the maximum P_{H_2O} was $93 \pm 2\%$ of saturation. (The temperature was controlled by a mechanical convection oven to a precision of better than ± 0.1 °C. P_{H_2O} as a percentage of saturation pressure was measured with a high-precision transducer and was verified by measuring the vapour pressure of pure water at 250 °C.)

(sample 6). Reaction with basaltic groundwater has the same effect as reaction with pure water (samples 10, 11), because the concentration of solutes in the water is insignificant compared with the concentration of soluble impurities and exchangeable ions in the bentonite.

Measurements of vapour pressure and water content of bentonite (unpublished work) have shown that the water is partitioned between water of hydration and vapour. Liquid water did not coexist with these samples at 250 °C.

To clarify the effects of liquid, vapour and water of hydration on bentonite, two experiments were performed, one in which liquid water was always absent and one in which liquid water was always present (to form a gel). In the first experiment, the bentonite was altered isothermally at a controlled partial pressure of water, 93% of saturation. Table 2 gives methods and results. The great reduction in swelling capacity shows that water of hydration and/or vapour was responsible for alteration. In the second experiment, water and bentonite were mixed in a ratio of 20:1 to form a gel. After reaction (samples 14 and 15, Table 1), the samples remained gelled, showing little or no loss of swelling capacity. Thus, reaction with water vapour, but apparently not with liquid, causes rapid, severe loss of swelling capacity.

The maximum alteration took place at water/clay ratios of roughly 0.15–0.25 at 250 °C (Table 1). This corresponds approximately to one to three absorbed water layers per 10-Å silicate layer in the montmorillonite structure (with allowance for up to 0.12 g water vapour in the autoclaves). Evidently, some water is necessary for the alteration, but alteration is slow or does not

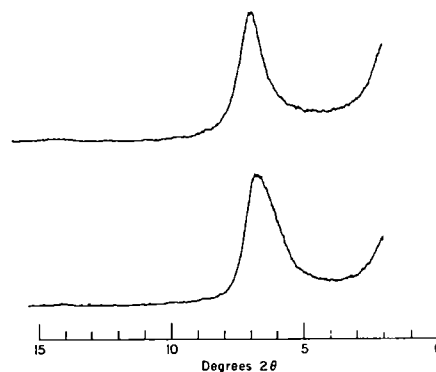


Fig. 1 X-ray diffraction of purified montmorillonite (Envirogel), unaltered (top) and altered (sample 12, bottom). Relative humidity controlled at 33% with saturated solution of $\text{MgCl}_2 \cdot 6\text{H}_2\text{O}$. Basal spacings: 12.4 Å unaltered, 12.5 Å altered, determined from four basal reflections. Complete conversion to the calcium form would shift the spacing to 15.4 Å, at $5.7^\circ 2\theta$ (ref. 18). $\text{CuK}\alpha$ radiation, General Electric diffractometer, slits: 1° entrance, medium resolution divergence, 0.2° receiving, $2^\circ 2\theta \text{ min}^{-1}$, time constant 2 s

take place in dilute systems, with low concentrations in the hydrated layers.

To determine whether the effect is reversible on contact with liquid groundwater, altered bentonite was reacted with a large excess of liquid groundwater. The swelling capacity of the product (sample 16) remained low. Therefore, the effect is not reversible over the timescale of the test.

The alteration is temperature-dependent, as shown by experiments at 250 °C, 200 °C and 150 °C (samples 9, 17 and 18). A large effect was observed as low as 150 °C.

The reduced swelling capacity corresponds to reduced uptake of water by hydrated layers of the montmorillonite structure. Unaltered montmorillonite expands greatly, in some cases from an average basal spacing of 10 Å in the dry state to over 100 Å in water^{2,15,16}. However, montmorillonite altered by water vapour at 250 °C expands only to a maximum basal spacing of 19 Å, which corresponds to three water layers per 10 Å silicate layer in the structure.

Except for the change in swelling behaviour, relatively little mineralogical change was observed. Both the altered and unaltered bentonite consist mainly of a dioctahedral smectite with an X-ray diffraction peak at 1.50 Å, and a basal spacing of 17 Å when treated with ethylene glycol. The reacted sample show evidence of slight uptake of exchangeable calcium from calcite, as shown by energy dispersive analysis in the scanning electron microscope (SEM) and as suggested by a small X-ray reflection at ~ 14.7 Å (at 33% relative humidity). However, a sample which was purified by sedimentation before reaction showed a similar reduction in swelling capacity (sample 12, Table 1) but much less uptake of calcium, as determined by analyses for exchangeable Na and Ca (Table 3), by analysis of single particles in the SEM, and as suggested by X-ray diffraction at controlled humidity (Fig. 1). Evidently, the change in swelling behaviour is not due to extensive exchange of calcium for sodium. Alteration was accompanied by significant fixation of magnesium (Table 3), but it is questionable whether the rather small amount of magnesium can account for the change in behaviour.

The mechanism has not been determined, but it may be due to hydrolysis of exchangeable cations, to an attack on the octahedral layer, with release of aluminium into interlayer positions, or to oxidation of ferrous iron.

The alteration was apparently not due to reaction with the vessel materials. No reaction rim was observed, and minimal swelling capacity was observed after reaction in either stainless steel or Inconel vessels. Furthermore, no uptake of Ni or Cr was detected by elemental analysis in the SEM.

The observed rapid change in montmorillonite properties has important implications for the use of bentonite at high temperatures. If bentonite is exposed to moisture at temperatures

Table 3 Exchangeable ions in bentonite (mmol g^{-1}) before and after alteration

	Na (method 1)	Ca (method 1)	Ca (method 2)	Mg (method 4)
Untreated (purified Envirogel)	0.56	0.16	0.11	0.05
Treated (sample 12)	0.51	0.13	0.12	0.01

Method 1: Determined by leaching overnight with 1 M NH_4Cl . Solutions were analysed by inductively coupled plasma atomic emission spectroscopy (ICP). Analytical accuracy $\sim \pm 5\%$. Partial dissolution of calcite may increase Ca analyses. Method 2: Determined by washing with 60% ethanol in water (by volume) and then leaching with 0.5 M LiCl, 0.5 M CsCl solution in 60% ethanol (after Neal)¹⁷. Samples (0.15–0.21 g) were rinsed twice for 15 min each with 60% ethanol, leached for 12 h with LiCl, CsCl solution, washed with 10 ml ethanol/water, leached for 2 h with 10 ml LiCl, CsCl solution, washed with 10 ml ethanol/water, then 5 ml. Samples were centrifuged after each washing or leaching. Leach solutions were combined and analysed by ICP. All solutions were de-aerated and kept under N_2 before use. This method is reported to dissolve insignificant amounts of calcite¹⁷. Analytical accuracy $\pm 5\%$.

of 250 °C or higher, the permeability⁷ and the ability to swell and fill fractures will be greatly decreased. The effects observed here need to be addressed for safe design of nuclear waste repositories, and additional work on the mechanism of alteration may be required. Reduction of the swelling capacity also occurs at 150 °C and may occur at still lower temperatures, especially after long time periods. Bentonite used to fill tunnels and drifts in nuclear waste repositories may reach high enough temperatures to be adversely affected by water vapour.

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Spin-dependent electron scattering from optically active molecules

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The origin of the isomeric purity of biomolecules has been extensively discussed¹. In particular, it has been suggested that spin-polarized electrons or positrons emerging in the β -decay of radioactive nuclei may have led to a preferential destruction of one of the two isomers at an early stage of evolution and that this process tipped the initial balance of abundance of the two self-replicating isomers². As experimental tests of this hypothesis have remained largely inconclusive^{3,4}, it seemed useful to investigate the spin dependence of electron scattering from optically active molecules under the simplest possible conditions. Here we describe an experiment which indicates that spin-polarized electrons, like polarized light, when scattered from optically active molecules, can 'distinguish' between right-handed and left-handed isomers.

The application of fundamental conservation principles has shown that the 'handedness' of target molecules leads to spin-

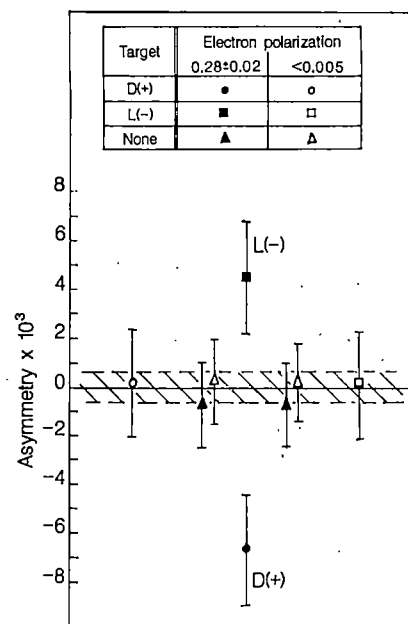


Fig. 1 Measurements of the electron polarization and beam attenuation with and without camphor and alternating the load in the target cell between the D(+) and L(-), respectively. Those obtained with camphor using polarized incident beams ($P = 0.28 \pm 0.02$) are marked D(+) and L(-), respectively. The remaining points were obtained with unpolarized incident beams ($P < 0.005$) or without camphor. The error bars indicate 1 s.e. The results obtained with unpolarized electrons or without camphor were also combined. The standard error of their weighted mean defines the hatched belt between the broken lines.

dependent scattering phenomena that are completely analogous to optical activity and circular dichroism⁵. Our objective here was to measure the attenuation of longitudinally polarized electron beams passing through a vapour of optically active molecules. This attenuation can be calculated in terms of two elastic forward-scattering amplitudes, one spin-independent, f , and the other chirality-dependent, h . The sign of h changes with the handedness of the molecules; $h = 0$ for non-chiral molecules. If I_0 is the incident beam intensity, P its longitudinal polarization, ρ the vapour density, z the path length, and the electrons undergo single scattering only, then the transmitted intensity is

$$I = I_0 \exp(-q\rho z) [\cosh(q\rho z X) - P \sinh(q\rho z X)] \quad (1)$$

where $q = (4\pi/k) \text{Im}(f)$ is the total spin-independent scattering cross-section and $X = \text{Im}(h)/\text{Im}(f)$. X measures the chirality-dependent cross-section in units of the spin-independent one, I_m denotes the imaginary part and k is the electron wave number.

If the transmitted intensity is measured for a pure isomer at a given target density and a given electron polarization, alternately parallel and antiparallel to the beam axis, the two output intensities, $I(P)$ and $I(-P)$, yield an intensity asymmetry,

$$A(P) = [I(P) - I(-P)]/[I(P) + I(-P)] \\ = -P \tanh(q\rho z X) = -P q \rho z X \quad (2)$$

to first-order approximation.

Thus, the following predictions can be made. (1) For a given isomer at a fixed magnitude of the longitudinal electron polarization, the magnitude of the asymmetry increases linearly with the sample density. (2) The asymmetry changes sign if the handedness of the sample is reversed. (3) The asymmetry vanishes if either the electron beam is unpolarized or the sample is a racemic mixture.

The first experiment, designed on the grounds of general symmetry considerations and using D(+)-camphor molecules as target and 25 eV initially unpolarized electrons, remained inconclusive in that it could establish only an upper limit to the in-plane polarization of the scattered electron⁶. Gidley *et al.*⁷ investigated asymmetrical positronium formation in optically

active molecules using slow spin-polarized positrons and found a positive effect in one case but none in two others.

The apparatus used in the present experiment consists of three stages: a GaAs polarized electron source⁸, an electron polarimeter used in a transmission mode to monitor the incident beam intensity and electron polarization⁹, and an electron spectrometer, adapted from the scheme introduced by Simpson¹⁰. The scattering target was camphor vapour at room temperature, and the experiment was carried out at 5 eV energy. Simultaneous measurements of the electron polarization and beam attenuation consisted of 'runs' taken in turn with and without camphor and alternating the load in the target cell between the D(+) and L(-) isomer, respectively. The results displayed in Fig. 1 were obtained by combining the results of corresponding runs.

Figure 1 shows, first, that in the absence of camphor and in the case of unpolarized incident electrons the observed asymmetry vanishes and, second, that for the two isomers, when electrons beams of the same polarization are used, the asymmetry is of different sign. The measured values are different from zero by 2 s.e. or more; the magnitudes of the asymmetries agree within their error margin. Thus, we conclude that the results confirm the existence of an 'electron optic dichroism' with a confidence limit exceeding 95%.

The experimental results allow a tentative estimate of the ratio $Im(h)/Im(f)$. In typical working conditions, camphor in the sample cell attenuated the beam by a factor of 4–5. As our equipment could not give an absolute measurement of the sample pressure, only relatively crude tests were performed to see that up to this level of attenuation single scattering conditions prevailed. Thus, from equation (2)

$$A = -P \ln(I_0/I) \quad Im(h)/Im(f)$$

and hence, with the measured values $P = 0.28$, $4 < I_0/I < 5$ and $A > 10^{-3}$, one finds

$$X = Im(h)/Im(f) > 2 \times 10^{-3}$$

The mechanism of the interaction involved is unknown. There are no theoretical estimates of X , although there are estimates of the related quantity $Re(h/f)$, where Re denotes the real part. Based on the 'helicity density' model, Rich *et al.*¹¹ estimated for camphor at low electron energy $Re(h/f) \sim 10^{-5}$. Hayashi¹² calculated the in-plane component of the spin polarization of initially unpolarized electrons scattered elastically by optically active molecules (1,3-dimethylallene) at 50 eV and above: $Re(h/f) < 5 \times 10^{-7}$.

We stress that the ratio X can be significantly different from $Re(h/f)$. More importantly, neither of the theoretical calculations quoted takes account of possible resonance scattering, which may increase the effect by orders of magnitude. At the energy at which the present experiment was conducted (5 eV), the possibility of resonance effects cannot be ruled out. It is suggestive that the optical absorption of photons about this energy certainly involves pronounced broad resonance^{13,14}.

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A small coleoid cephalopod with soft parts from the Lower Devonian discovered using radiography

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During the past 50 years, X-ray examinations have greatly increased our knowledge of fossils. Modern radiographic methods, X-ray tubes with fine focal spots, high-resolution films and optimal exposure conditions have revealed details of the anatomy and general biology of ancient life as well as species and representatives of major groups hitherto unknown in the Lower Devonian^{1–5}. Most of this material came from the Hunsrück slate, where most fossils are converted to pyrite, FeS₂, giving a good contrast in the radiographs. Additional image processing may reveal even more details. While screening large quantities of black slate pieces, I have discovered a small teuthid coleoid showing soft parts (Fig. 1) which is the best-preserved specimen I have found during 20 years of investigation. This *Eoteuthis elfriedae* n.sp. is very similar to the living *Alloteuthis africana* of the Loliginidae, and is one of many unusual fossils yielded by this slate^{3–5}, only a few of which have been published. This specimen of *E. elfriedae* shows that *Alloteuthis*-like animals have not changed much over the past 400 Myr, and means that previous concepts of the appearance of such forms must be revised.

More than 100 gladii and cuttle bones of other teuthid coleoids have been recovered in the past 2 years from the Kaisergrube, an old mine near Gemünden in the Hunsrück. They all show a typical growth striation, but radiography reveals that none of them has soft parts. Similar striation has been observed⁶ in the



Fig. 1 a, Extant *Alloteuthis africana* (Gulf of Guinea, R/V Geromino Sta 2-730680, Smithsonian Institution). b, Radiograph of *A. africana* (Gulf of Guinea, R/V Geromino Sta 2-1910 from 730680, Smithsonian Institution). c, Radiograph of the Lower Devonian teuthid of the Black Slate of Bundenbach/Hunsrück, FRG (WS 3023). Scale bars, 10 mm. The radiographs were made with a Siemens Kristalloflex II unit in combination with a special water-cooled fine focus tube. The Agfa Millimask plates were used as photographic material which allow observation of details down to <5 μm. Intermediate negatives with enhanced detail contrast were made using a TV-microscope in combination with a Siemens Transicon image processing unit.



Fig. 2 The head of *Eoteuthis* showing the tentacles. The series of pyrite dots is visible. The jaw can be seen in the lower half. Scale bar, 10 mm.

Table 1 Description of *Eoteuthis elfriedae* n.sp.

Class	Cephalopoda
Subclass	Coleoidea BATHER 1888
Order	Teutkida NAEF 1916
Family	Loliginidae <i>sensu stricto</i> Steenstrup 1861. As only one specimen exists, the characterization of the genus must be the same as that of the species.
Species*	<i>Eoteuthis elfriedae</i> n. sp.
Holotype	Bavarian State Coll. Munich, No. 1984 I 100 (Radiographs: WS 3023); specimen showing soft parts on radiographs (Fig. 1)
Locality	Black slate mine Herrstein, Bundenbach, Hunsrück (FRG)

* The species is named in honour of Mrs Elfriede Stürmer, whose enthusiastic efforts have revealed hundreds of Lower Devonian coleoid cephalopod remains.

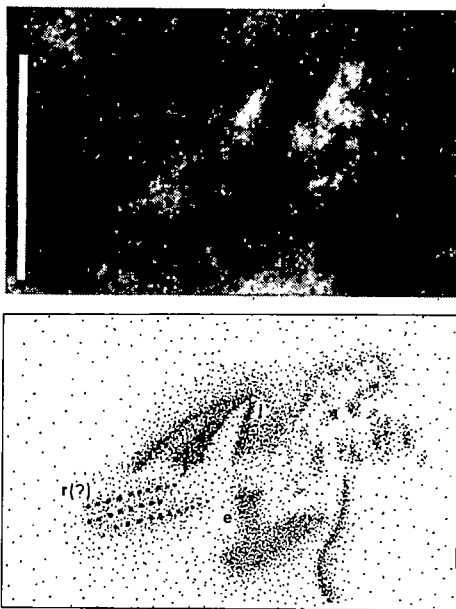


Fig. 3 The jaw of *Eoteuthis* after image processing: j, jaw; e, oesophagus; r, radula (?). Scale bar, 1 mm.



Fig. 4 The fin of *Eoteuthis* with concave posterior border after electronic image processing. Scale bar, 10 mm.

Table 2 First known evidence of cephalopod fossils

Cambrian	Ordovician	Silurian	Devonian	Carboniferous	Permian	Triassic	Jurassic	Cretaceous	Tertiary
							①		
				②					
			③						
④									

1, *Proteroctopus ribeti*; 2, *Jeletzkyia douglassae*; 3, *Eoteuthis elfriedae*; 4, Orthoceratid cephalopods.

fragmental prebelemnites, *Hyolithoconularia striata* found in the Lower Devonian of Morocco. A detailed account on the Hunsrück material will be published elsewhere.

The preservation of the soft part of a fossil is unusual but the Hunsrück slate contained favourable conditions for preservation^{4,5}: the organic sulphur compounds of the animal (or of sulphur bacteria) form pyrite, FeS₂, in the presence of iron ions in the interstitial water of the sediment. This pyrite is opaque

to X rays, so even a thin veil of FeS₂ at optimal exposure conditions produces a good contrast in the radiograph^{4,5}.

The body organization of this little teuthid, *E. elfriedae*, resembles that of the living *Alloteuthis* (Table 1) and seems to indicate that this type of cephalopod existed in the Lower Devonian. It is a good example of long survival in the fossil record.

This small teuthid cephalopod is similar to the extant *A.*

africana, with some well-preserved soft parts. There are eight or nine visible arms/tentacles, not definable in detail; the jaw is partly visible in the radiograph (Fig. 2) together with a fin which has a concave posterior border (Fig. 4). The total length of *E. elfriedae* is 83 mm, its maximum width 9 mm. The specimen is flat and has an estimated thickness of <0.5 mm; the original diameter of the more or less tubiform animal may have been ~6 mm. Some of the arms/tentacles have rows of little spots (suckers?) (Fig. 2). The jaws are visible just behind the tentacle bases (Fig. 3). The oesophagus seems to be below the tip of the beak (Fig. 3, e). To the left, between the beak and oesophagus are three rows of faint regular dots which may represent the radula (Fig. 3, r). One of a pair of lateral fins (Fig. 4) has a concave posterior border, as in the extant *Alloteuthis*⁷. The opposite fin is directly attached to the body and is thus invisible in the radiograph. Owing to the heavy pyritization of the body the gladius cannot be reconstructed. We must assume that the animal was buried very rapidly which prevented bacterial dissolution of the soft structures. Also, compaction must have taken place at an early state of diagenesis, flattening the body and the soft parts to <0.5 mm. The distortion of the soft parts as a result of this compaction makes it difficult to compare with living *Alloteuthis*-like animals. The process of pyritization is not fully understood. Observations on slate pieces from the Kaisergrube reveal hundreds of small cephalopods and tentaculites on one slab, and several states of FeS₂ exist side by side. My experiments on the fossilization process in the laboratory by putting *Cirolana* specimens from South Norway into different metal salt solutions produced a surprising result: radiographs made at 6-weekly intervals for the first year, then at 6-monthly intervals, show a 'fossilization' that remains constant after the first 6 weeks, so the experiment was concluded after 4 yr.

There are many tables⁸⁻¹² illustrating the ancestry of cephalopods, but because some points in such evolution charts are based on only a few moderately well preserved fossil specimens, only general statements should be made until we know more about fossil and modern gladii (see ref. 13 for a critical analysis). Table 2 shows that a fully developed octopus, *Proteroctopus tibeti*¹, existed in the Jurassic; Johnson and Richardson² found a 10-armed cephalopod *Jeletzkyia douglassae*, in the Mazon Creek fauna from the Middle Pennsylvanian of Illinois. Radiography clearly reveals the complete tentacular crown and fragile shell of this unique fossil. Double rows of hooks on the arms are well preserved. *E. elfriedae* is a highly developed teuthid of the Lower Devonian, and the ectocochleate nautiloids go back to the Cambrian¹⁴. If we consider the timescale and the fact that the fossil record shows few closely related forms, then more questions arise, especially as to the origin of these animals. How long did it take to evolve from a primitive nautiloid to *Eoteuthis*, and where might one hope to find connecting forms?

I thank Professor G. L. Voss for the first hint that the fossil may be an *Alloteuthis* and for helpful discussions. Financial support for the Hunsrück research by the Volkswagen Foundation and the German Research Society is acknowledged. I also thank Dr C. F. Roper and Dr Jeletzky, for many discussions; and especially Dr C. F. E. Roper for specimens of extant *Alloteuthis* animals for comparison radiographs.

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Biogenic fluxes of carbon and oxygen in the ocean

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Rates of oxygen utilization (OUR) at depth in the ocean have been interpreted as showing that rates of carbon fixation by phytoplankton, as estimated by ¹⁴CO₂ assimilation *in vitro*, must be in error¹. The oxygen is consumed in the decomposition of organic matter sinking from the photic zone: there is a stoichiometrically equivalent flux of nitrate from the deep water towards the surface². For comparison with the ¹⁴C data, it is conventional to extrapolate OUR to total equivalent phytoplankton production through a constant factor *f*, the ratio of nitrate-based production (*P*_{new}) to total production (*P*_t) as defined by Dugdale and Goering³. The alternative hypothesis, that scaling up OUR by a constant factor *f* is inappropriate, has not been examined in detail. We show here that *f* is variable in space and time for most provinces of the ocean. Furthermore, we show that in nitrogen-limited systems, such as the pelagic of the open ocean, *P*_t and *f* should be positively correlated. Applying these results to data from the Sargasso Sea, we find that the carbon fluxes estimated by ¹⁴C assimilation are consistent with the oxygen fluxes estimated by OUR. The conclusion is of profound importance for understanding the major biogeochemical cycles of the ocean.

Alternative methods for measuring the same ecological rate do not necessarily refer to the same time-scale. Accumulation of oxygen in the photic zone of the ocean⁴ or its consumption at depth^{1,5} provide estimates of phytoplankton production with a characteristic timescale of months to years. Measurements of carbon fixation by *in vitro* incubation have a characteristic time-scale of the same order as the incubation time, ≤24 h. We have pointed out^{6,7} that extreme caution should be taken when comparing data sets whose intrinsic timescales are not compatible. The one has to be averaged to conform with the other, and we usually have little information on which to base the averaging in time. Moreover, OUR calculations imply some averaging over depth, whereas ¹⁴CO₂ assimilation is measured at discrete depths.

For an arbitrary depth stratum, one need not necessarily expect that the carbon and oxygen fluxes will balance when averaged over some arbitrary time interval. Within the variance between years (which can be appreciable⁸), the natural biological timescale at which the carbon and oxygen fluxes can be expected to balance is the annual one. One of the very few

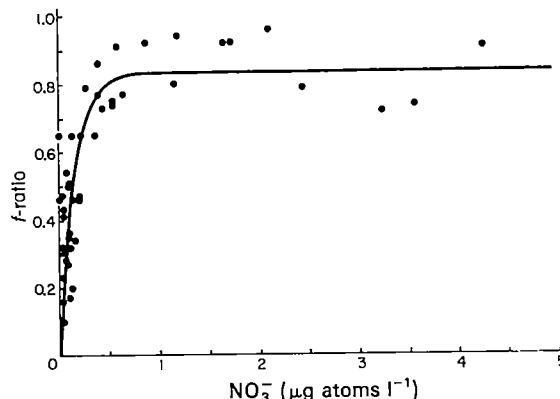


Fig. 1 Relationship between *f* and ambient NO₃⁻ concentration using the depth profile data of ref. 25 and the seasonal data of ref. 26. Data for which ambient NH₄⁺ concentrations exceeded 0.1 μg atoms l⁻¹ were omitted. Data fitted to an exponential model: $f = f_{\max}(1 - e^{-(\alpha \text{NO}_3^-/f_{\max})})$, for which $f_{\max} = 0.83 \pm 0.08$ and $\alpha = 5.48 \pm 0.77$.

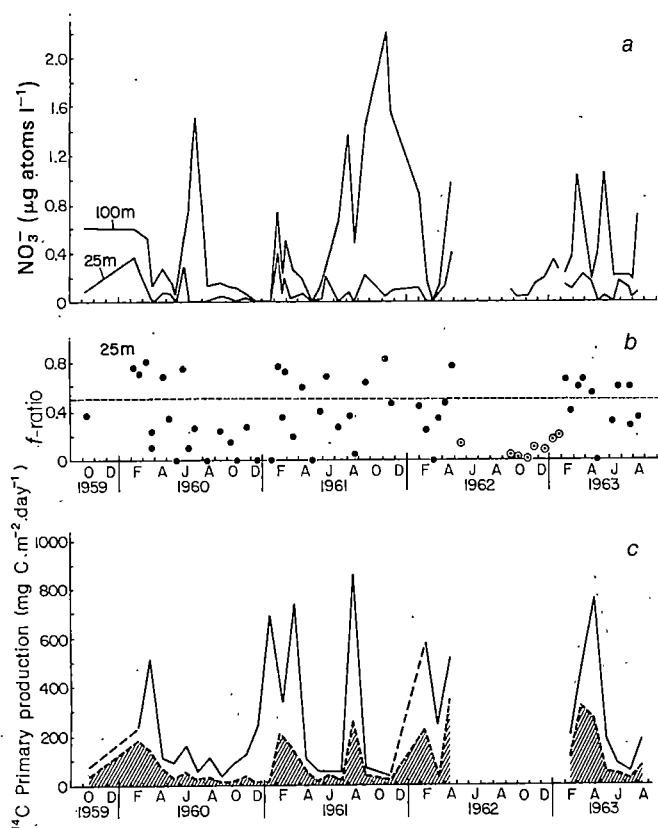


Fig. 2 Time-series data of: *a*, ambient NO_3^- concentrations; *b*, f computed from the model given in Fig. 1; and *c*, total ^{14}C , and new primary production (P_{new}) at station S, Bermuda; integrated 0–100 m. New production (P_{new}) estimated as fP_T where P_T is assumed to be equivalent to ^{14}C results. In *b* \odot are f values determined directly from ^{15}N measurements (ref. 3): these data appear to be fully consistent with the trends shown by the indirectly determined values of f .

oceanic data sets having anywhere near sufficient resolution in time that reliable annual means of primary production and OUR can be calculated is that from station S in the Sargasso Sea⁸. From these data, Jenkins and Goldman⁵ compute an OUR at depth of the order of $5 \text{ mol m}^{-2} \text{ yr}^{-1}$. We now consider how this estimate of new production should be scaled up to give total production.

The conventional procedure¹ is to apply to the annual new production a constant factor $\bar{f} \approx 0.05$ said to be characteristic of oligotrophic ocean water². An alternative procedure, adopted here, is to estimate $f(z, t)$ for each depth z for all times t during the year.

Normally f is estimated from measurements of the *in vitro* assimilation rates of $^{15}\text{NO}_3^-$ and $^{15}\text{NH}_4^+$. There are very few such data for the Sargasso Sea. However, provided the concentration of ammonia is low, $\leq 0.1 \mu\text{M}$, such that neither the uptake of nitrate nor its enzymatic reduction are suppressed, f can be related to ambient nitrate concentration (Fig. 1). We use this dependence to estimate $f(z, t)$ from the nitrate concentrations reported at station S (Fig. 2). It is found that f is not constant, but varies between 0 and 0.84.

We now state an important inequality for nitrogen-limited systems:

$$\iint f(z, t) P_T(z, t) dz dt \geq \bar{f} \iint P_T(z, t) dz dt \quad (1)$$

where

$$\bar{f} = \frac{\iint f(z, t) dz dt}{\iint dz dt}$$

In other words, P_{new} estimated by the weighted integral on the left-hand side of equation (1) will always exceed P_{new} estimated

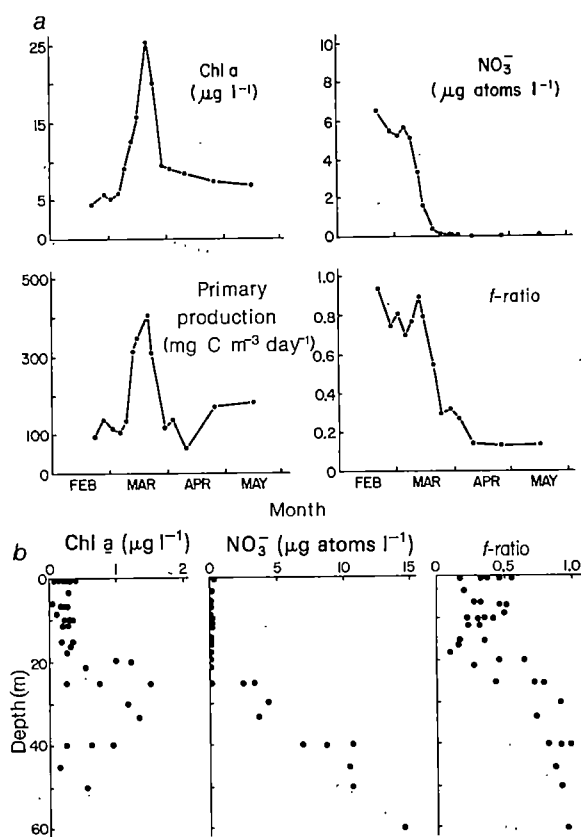


Fig. 3 Relationship between f and ambient NO_3^- concentration: *a*, during the decline of the spring phytoplankton bloom in a temperate coastal embayment (Bedford Basin, Nova Scotia); and *b*, in depth profiles on the continental shelf of the middle Atlantic Bight in summer. Here, f is derived directly from $^{15}\text{NO}_3^-$ and $^{15}\text{NH}_4^+$ uptake measurements.

by the right-hand side, mean value approximation. This is because $P_i(z, t)$ and $f(z, t)$ will have a finite (positive) covariance where nitrogen is the limiting resource. That is, a local pulse in available nitrate will result in a local increase in f at the same time²⁷ as it increases the local magnitude of P_i (Figs 2, 3).

Applying the weighted integral to the ^{14}C data for station S, one finds that, on an annual basis, new production is 31% of total production (Table 1). The ^{14}C data show an average production of $6.8 \pm 1.9 \text{ mol C m}^{-2} \text{ yr}^{-1}$, separable now into $2.1 \pm 0.58 \text{ mol C m}^{-2} \text{ yr}^{-1}$ of new production and $4.7 \pm 1.3 \text{ mol C m}^{-2} \text{ yr}^{-1}$ regenerated production ($P_T - P_{\text{new}}$). Applying photosynthetic quotients of 1.8 to the new production and 1.25 to the rest⁹, we find the oxygen equivalent of the ^{14}C results to be $9.7 \pm 1.9 \text{ mol O}_2 \text{ m}^{-2} \text{ yr}^{-1}$, of which $3.8 \pm 1.0 \text{ mol O}_2$ is new production. Jenkins and Goldman⁵ found an OUR at depth to be in the range $4.1\text{--}5.9 \text{ mol O}_2 \text{ m}^{-2} \text{ yr}^{-1}$ depending on the corrections applied for diffusive losses and abyssal respiration. It does not appear, therefore, that there is any significant difference between the two independent indices of new production. And we certainly do not find that the respiration at depth (P_{new}) exceeds the primary production at the surface (P_T), as was claimed for the subtropical gyre of the North Atlantic, and interpreted as showing that the ^{14}C results must be in error¹.

In fact, adopting a small and constant \bar{f} when P_{new} is as high as $5 \text{ mol O}_2 \text{ m}^{-2} \text{ yr}^{-1}$ leads to a contradiction in Fig. 2a of ref. 2, for then the implied estimate of P_T is $\approx 100 \text{ mol O}_2 \text{ m}^{-2} \text{ yr}^{-1}$, which would require an asymptotically high value for \bar{f} (≈ 0.5). We have shown that the contradiction stems from an improper selection of \bar{f} rather than from a discrepancy between the ^{14}C results and the true values of total production. Indeed, putting the effective annual $\bar{f} = 0.31$ as calculated here into equation (1) of ref. 2 yields $P_T = 124 \text{ g C m}^{-2} \text{ yr}^{-1}$, not grossly at variance with the mean annual ^{14}C value of $82 \pm 23 \text{ g C m}^{-2} \text{ yr}^{-1}$ (Table

Table 1 Mean monthly and annual ^{14}C primary production rates from Station S, 1959–63

Month	Primary production (g C per m ² per month)		
	P_T	P_{new}^*	f
1	12.2 (12.4)	1.2 (1.5)	0.10
2	10.2 (5.1)	5.4 (1.6)	0.53
3	14.9 (6.1)	4.8 (3.4)	0.32
4	11.3 (9.7)	5.5 (4.4)	0.49
5	3.3 (2.2)	0.8 (0.6)	0.24
6	2.8 (1.5)	1.2 (0.3)	0.43
7	1.7 (0.1)	0.6 (0.1)	0.35
8	11.8 (12.5)	3.5 (3.6)	0.30
9	1.6 (0.5)	0.7 (0.5)	0.44
10	2.5 (0.4)	0.6 (0.4)	0.24
11	2.3 (1.8)	0.7 (0.3)	0.30
12	7.5 (4.4)†	0.2 (0.1)†	0.03
Annual	82.1 (22.2)‡	25.3 (7.0)‡	0.31§

Monthly rate = mean daily rate $\times 30$. Data are given ± 1 s.d.

* $P_{\text{new}} = \int P_{\text{new}}(z, t) dz$ averaged for the month, where $P_{\text{new}}(z, t) = P_T(z, t) \times f(z, t)$.

† Variance estimated.

‡ The measures of dispersion given are ± 1 s.d. of the annual totals computed on the basis of the standard deviations of the monthly averages; they are not intended to be explicit statements of the confidence intervals at any particular probability level.

§ Annual P_{new} divided by annual P_T . Annual figures are based on some 51 sampling dates.

1). Note also that these two values are quite independent of each other.

Another problem with the extrapolation of high values of total production through division of new production by a small value of f is that the implied efficiency of photosynthesis is too great to accept. It can be shown that extrapolation of the OUR estimates of P_{new} to P_T through application of an f ratio ≈ 0.5 is equivalent to a photosynthetic efficiency $\geq 6\%$, a value too high to be believable as an annual average¹⁰.

Evidence is accumulating that the distributions of biological properties in the ocean are highly variable in space and time. We conclude this from sediment trap data¹¹; from satellite data¹²; from samplers deployed from a moving ship¹³; from theoretical physical oceanography¹⁴; and from theoretical ecology¹⁵. Superimposed on this variance in the Sargasso Sea is a strong seasonal cycle in both primary production and nutrient availability¹⁶; for example, the four months January–April account for $\approx 60\%$ of the annual assimilation of $^{14}\text{CO}_2$ (Fig. 4).

It therefore becomes increasingly untenable to speak about a particular ocean province, and certainly not the Sargasso Sea, as being 'oligotrophic'. Rather, a given oceanic province can be expected, locally in $(x, y, z \text{ and } t)$, to manifest a range of characteristics from apparent extreme oligotrophy to eutrophy. For example, judging by the f ratio alone, a temperate, coastal embayment can appear to change its trophic status from eutrophic to one characteristic of the open-ocean stereotype within the space of a few weeks, if a sufficiently detailed time-series is available (Fig. 3a). Local or transient fluctuations away from oligotrophy will account for a high proportion of the annual primary production. Moreover, most of the production in these events will be new production. The effective f ratio for the year (proportion of the total annual carbon fixation that is based on nitrate) will, therefore, be much higher than has previously been supposed.

The open ocean is still heavily undersampled with respect to primary production. For example, the water column primary production rates for Station S have to be computed from data collected at only three depths. In the open ocean, the distribution of phytoplankton is highly non-uniform in depth¹⁷, and the existence almost everywhere in the ocean of a deep chlorophyll maxima is well established¹⁸. If more sampling depths were

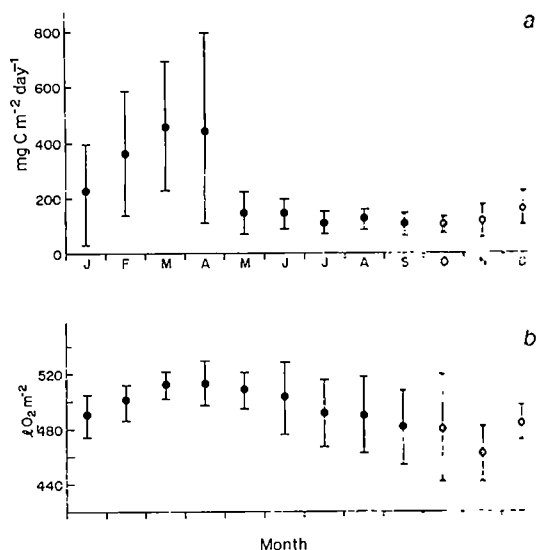


Fig. 4 Long-term averages (± 1 s.d.) of ^{14}C primary production (a) and dissolved O_2 (b) concentrations at Station S, Bermuda. These values represent a longer time-series (1957–63) than those shown in Fig. 2.

available, the estimate of depth-integrated production would probably increase. A similar tendency for the mean rates to increase can be expected as the resolution of sampling is improved in time and in the (x, y) plane.

We conclude that in a nitrogen-limited ocean ecosystem, local events (positive anomalies) in the nitrate field account for a disproportionately high fraction of the annual signal in primary production. During these events, the margin between new and total production narrows. On the other hand, the factor converting carbon fixation to oxygen evolution is at its highest. Taken together, these points are sufficient to show that the available flux data for carbon and oxygen in the Sargasso Sea are not internally inconsistent. It is one more example of the crucial importance of giving at least as much emphasis to the variances as to the means in discussing the dynamics of nonlinearly coupled systems¹⁹. The implied picture of the pelagic ecosystem (intermittent nitrate supply; new production one-third of total) is at variance with that presented by Goldman²⁰ where production is controlled by 'oases of regeneration' associated with particulate aggregates, and where 'spinning wheel' metaphor characterizes the supposed, rapid rate of nutrient recycling.

These conclusions are of importance not only for our understanding of the pelagic ecosystem but also for incorporation into budget calculations for the great biogeochemical cycles of the ocean^{21,22}. The conclusions have a direct bearing on current discussions of the possible role of the ocean biota as a control on the concentration of CO_2 in the atmosphere, and thus bear indirectly on the prediction of the future climate of the Earth^{23,24}.

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A structurally preserved magnolialean fructification from the mid-Cretaceous of Japan

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Fossil evidence throwing light on the origin and diversification of angiosperms (flowering plants) has been increasing rapidly over the past 10 years. Most palaeobotanists accept that angiosperms first diversified in the Barremian stage of the Cretaceous, but this is based mainly on strato-phenetic morphological studies of palynomorphs and vegetative organs¹⁻³. Reproductive organs such as flowers and fructifications, which are vital to a full understanding of angiosperm evolution, are rare and have only recently been described in any detail from Cretaceous rocks⁴⁻¹¹, and even these are generally preserved as compressions or as fusinized remains. Here I report a permineralized angiosperm floral axis bearing conduplicate carpels from mid-Cretaceous sediments of Hokkaido, Japan. Its structure most closely resembles that of extant Monimiaceae and Austrobaileyaceae of the Magnoliales, particularly in the concave receptacle, but it shows several features considered more primitive than those occurring in any extant family. The fossil extends and clarifies knowledge of the structure and evolution of early angiosperms and is the first report of a permineralized angiosperm fructification of Cretaceous age.

The fructification was collected by Mr Masaharu Kasai in a tributary of the Sankebetsu River, Haboro district of Hokkaido, in Cretaceous shallow marine sediments of the Upper Yezo Group. The ammonite *Mesopuzosia pacifica* Matsumoto in the same calcareous nodule indicates a Turonian age.

The specimen has a woody peduncle, 25 mm long and 4 mm in diameter, with an attached globose floral structure ~25 mm in diameter (Fig. 1a). The floral structure is composed of many foliicles (Figs 1b, 2), and reconstruction (Fig. 2b) illustrates that

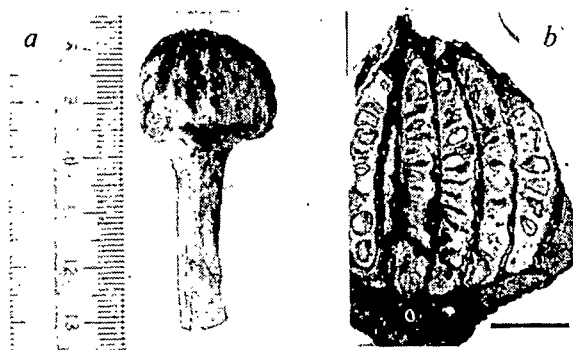


Fig. 1 a, Side view of the fossil fructification showing attachment to a long peduncle. Scale in cm. b, Longitudinal section showing concave receptacle and falcate foliicles containing ovules. Follicles from right to left correspond to those in Fig. 2b between the two parallel lines and numbered 18, 11, 6, 4, 2 and 3. Scale bar, 0.5 cm.

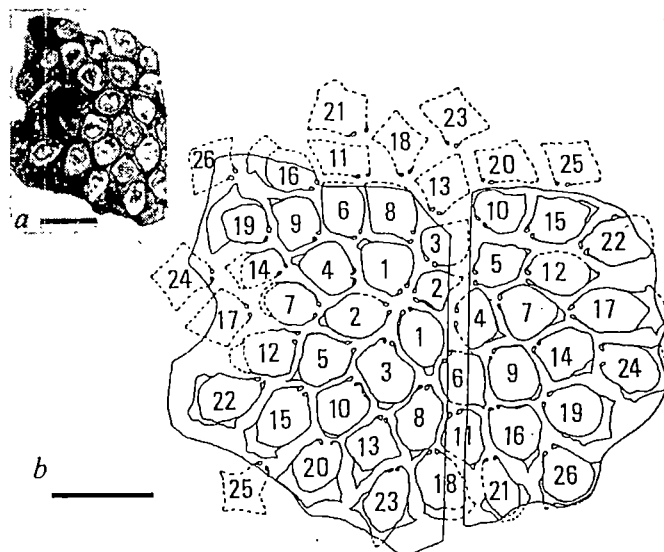


Fig. 2 Cross-sections of the fructification. The upper part of the figure corresponds to the surface shown in Fig. 1. a, Section of the specimen corresponding to the left half of b. b, Diagrammatic reconstruction of foliicle arrangement. Numbers show pairs of foliicles in a helically decussate arrangement. Scale bars, 0.5 cm.

the arrangement of the foliicles is intermediate between decussate and helical.

The median longitudinal section in Fig. 1b shows that the foliicles were borne on a concave receptacle. Each foliicle is 17 mm long × 3.1 mm wide and falcate, curved slightly towards the adaxial surface. They are sessile or have a short stalk and taper towards their apices. An adaxial longitudinal slit starts 1 mm from the base of each foliicle and runs nearly to the apex. A pair of marginal ribs occurs alongside this slit, bearing abundant unicellular, simple trichomes (Fig. 3). The slit evidently represents the stigmatic crest of a conduplicate carpel. Paracytic stomata occur on the external wall of the foliicle.

In the foliicle a pair of adaxial veins passes through the marginal ribs along the stigmatic crest. An abaxial vein runs right up to the apex of the foliicle, producing an unknown number of lateral bundles, and then curves to the adaxial surface, where it ends blindly about 0.5 mm below the point of incurving.

Most of the receptacle is infilled with sandstone matrix, but its base is preserved and shows many bi-collateral bundles arranged in a ring. There is no direct evidence that stamens or petals were attached, but bundles occur at the base of the receptacle which do not enter foliicles and are directed to the receptacle periphery. These may represent traces to stamens or perianths.

There are at most 15 ovules per foliicle attached alternately along the adaxial stigmatic crest, and therefore having marginal placentation. The funicles are short and the ovules bitegmic (Figs 3, 4). The outer integument comprises a thick, sclerotic outer layer and a thin inner one which develops a thick parenchymatous cushion around the micropyle. The inner integument is only one cell thick and is generally very thin, but the cells surrounding the micropyle are elongated radially, making the layer thicker.

A globose structure of unknown function is present between the micropyle and funicle (Fig. 4a). It seems to be some kind of aril, but further discussion is precluded at present because so little is known about the ovuliferous structures of comparable living angiosperms. No palynomorphs were recovered.

Secondary xylem is well developed at the base of the peduncle, and mucilage cells surrounded by epithelial cells are abundant in the pith. Distal to the peduncle the vascular bundles subdivide to form a eustele; large oil cells are distributed in the cortex of the peduncle, each containing a globose, yellowish-brown, transparent body. Such cells also occur in the foliicle walls.



Fig. 3 Cross-section of adaxial opening of a follicle. Arrows indicate simple trichomes. Note the short funicle (f) extending from the marginal rib. Scale bar, 50 μ m.

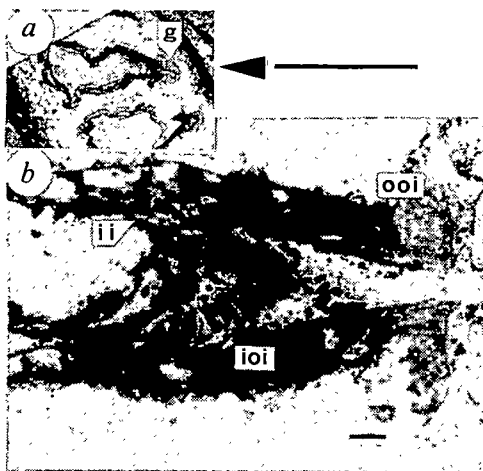


Fig. 4 Ovule structure. *a*, Median longitudinal section of two ovules. Note the small globose structure (*g*) attached to the micropylar end of each ovule. Scale bar, 0.2 cm. *b*, Details of the micropylar end of an ovule. ooi, Outer layer of outer integument; loi, inner layer of outer integument; ii, inner integument. Scale bar, 50 μ m.

Based on the polycarpelous floral structure of numerous conuplicate carpels and the relatively primitive wood structure composed of mainly scalariform vessels with scalariform perforation plates, the fossil is assigned to the Magnoliales. Monimiaceae and Austrobaileyaceae are the most closely comparable families, having similar concave receptacles with apocarpous carpels. However, the carpel of these families is more specialized in lacking a prominent stigmatic crest. Carpels of Monimiaceae have a single ovule, whereas those of Austrobaileyaceae have 8–14 (ref. 12). Because the present fossil has many primitive characteristics, it is difficult to assign it to a modern family.

The carpel, with a long stigmatic crest and a large number of ovules, is unknown in any living magnoliatean, but the compressed flower *Archaeanthus* Dilcher and Crane^{7,10} from the mid-Cretaceous of Kansas is comparable in this feature and also in having oil cells in the carpel walls. *Lesqueria* Crane and Dilcher and other unnamed fruits also from mid-Cretaceous of Kansas and Texas¹¹ have comparable follicle structures. The fossils differ, however, in the shape of the receptacles. All the above flowers and fruits other than the unnamed one have either elongate or ovoid receptacles on which the follicles are helically arranged, more like a living *Magnolia* flower. Note that in the early stages of magnoliatean evolution, flowers evidently existed that had two quite different forms of receptacles, both of which bore carpels possessing similarly primitive features.

Although the fossil record of the early angiosperms is limited, it demonstrates through time and space the characteristics that

have existed in the evolutionary history of angiosperms. More detailed knowledge of the Cretaceous diversification of angiosperms requires further comparative studies of both fossil and living angiosperm flowers. For the present specimen to be compared with flowers or fruits of living Magnoliales, further knowledge is needed about the ontogeny and anatomy of carpels and receptacles, ovule structure, carpel arrangement and wood anatomy of living members. The use of cladistic analysis may provide a more objective evaluation of such comparative data^{3,13,14}. This method may be one of the more effective ways of answering such basic questions as what the angiosperms are, when and from what kind of ancestors they may have originated, and how they have diversified.

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Abolition of specific immune response defect by immunization with dendritic cells

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Murine cytotoxic T (T_c)-cell responses to various antigens are controlled by immune response (*I*r) genes mapping in the major histocompatibility complex (*H*-2). The genes responsible are those encoding the class I and class II *H*-2 antigens^{1–4}. The *H*-2 *I*-A^b mutant mouse strain bm12 differs from its strain of origin, C57BL/6 (*H*-2^b), only in three amino acids in the *I*-A^b class II *H*-2 molecule^{5–7}. As a consequence, female bm12 mice are T_c -cell nonresponders to the male antigen H-Y and do not reject H-Y disparate skin grafts⁸. We now report that bm12 mice generate strong H-Y-specific T_c cells following priming *in vivo* and restimulation *in vitro* with male bm12 dendritic cells (DC). Female bm12 mice primed with male DC also reject male skin grafts. Furthermore, we demonstrate that only responder cell populations containing a mixture of L3T4⁺ (T-helper (T_h) phenotype) and Lyt 2⁺ (T_c phenotype) T lymphocytes generate H-Y-specific T_c cells. These data imply an essential role for T_h cells, activated by DC as antigen-presenting cells (APC), in changing H-Y-nonresponder bm12 mice into H-Y responders. Priming and restimulation with DC allows the triggering of a T-cell repertoire not demonstrable by the usual modes of immunization. This principle might be used to overcome other specific immune response defects.

Effective T_c -cell responses to at least some antigens are elicited only in mice possessing 'responder' alleles at both class I and

class II *H-2* loci. Thus, C57BL/6 (B6) mice generate a strong *H-2D^b*-restricted *T_c*-cell response to H-Y by virtue of the presence of the *I-A^b* and *D^b* 'responder' alleles^{2,3}, illustrating effective *T_h* and *T_c* precursor activation pathways, respectively.

Previous work has demonstrated that nonresponsiveness of bm12 mice to H-Y is caused by a defect in the *T_h* repertoire³. Recently, dendritic cells were shown to be extremely effective in presenting antigen to *T_h* lymphocytes⁹⁻¹³. We therefore investigated whether it was possible to overcome the specific inability of bm12 mice to respond to H-Y by using dendritic cells as APC. To this end we used male DC to immunize female bm12 mice, and to restimulate their spleen cells *in vitro*. The ability of DC to activate H-Y-specific *T_c*-cell precursors was compared with that of bm12 normal spleen cells (NSC) and lipopolysaccharide (LPS)-stimulated bm12 lymphoblasts. Spleen cells from bm12 female mice primed by intraperitoneal (i.p.) injection of 10^7 syngeneic male NSC failed to mount an H-Y-specific *T_c* response, irrespective of the type of cell used for *in vitro* restimulation (Fig. 1); even addition of the growth factor interleukin-2 (IL-2) during the *in vitro* restimulation did not result in the generation of H-Y-specific *T_c* cells. In agreement with previous results^{3,8}, all other tested immunization procedures including intravenous (i.v.) and subcutaneous footpad immunization¹⁴ with NSC failed to yield H-Y-specific *T_c* responses (data not shown). After priming *in vivo*, followed by restimulation *in vitro*, with male bm12 LPS blasts, a very weak H-Y-specific *T_c* activity was elicited. Strikingly, however, bm12 spleen cells generated strong H-Y-specific *T_c* activity when syngeneic male DC were used both for priming and for *in vitro* restimulation. Priming with DC followed by restimulation with NSC yielded negative results. In this case, however, addition of IL-2 resulted in an H-Y-specific *T_c* response (Fig. 1).

The biological significance of these findings was evaluated further using skin grafting experiments. Unprimed B6 mice or B6 mice primed with B6 male NSC all rejected male skin grafts with mean survival times of ± 23 days (Table 1). Male skin grafts on unprimed bm12 females or bm12 females primed with syngeneic NSC showed mean survival times of >120 days. McKenzie *et al.*¹⁵ showed that female bm12 mice were unable to reject male skin grafts unless they had received prior footpad priming with male NSC; the rejection rate was significantly slower than in other primed strains. As stated previously, we were unable to demonstrate H-Y-specific *T_c* responses in bm12 mice after footpad immunization³. In contrast, priming of bm12 females with male DC led to a dramatic decrease of male graft survival (mean time for graft rejection = 22 days; Table 1). In fact, bm12 mice primed with DC rejected their skin grafts as quickly as B6 mice primed with NSC (group 2; Table 1). The difference in survival time between the DC-primed bm12 mice and the control group was highly significant ($P < 0.001$; compare groups 6 and 4 in Table 1). All graft rejections were H-Y-specific, because all animals were also grafted with female grafts, which all survived indefinitely (>120 days). The important *in vivo* role of DC in graft rejection has also been demonstrated in allograft models^{17,18}.

Further experiments indicated the necessity for H-Y antigen to be present on the DC used for priming, because immunization

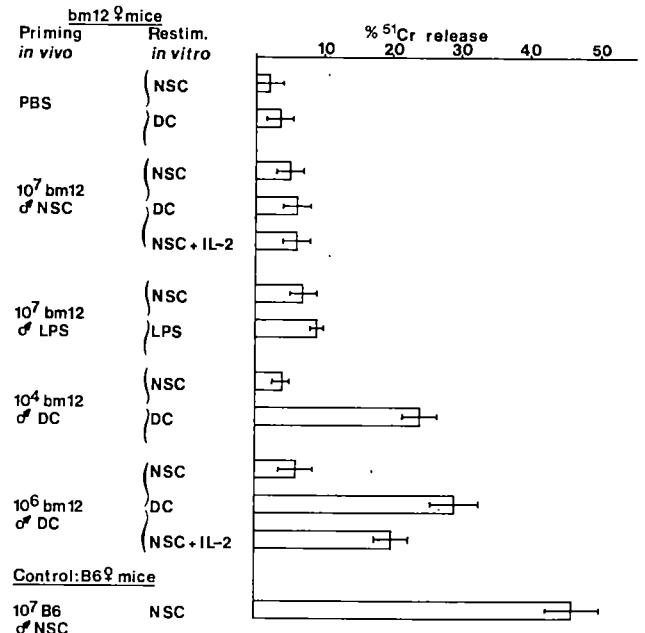


Fig. 1 H-Y-specific *T_c* response of bm12 *I-A* mutant mice following various modes of immunization.

Methods. Dendritic cells were isolated as described by Steinman *et al.*¹⁶. Briefly, spleen cells were spun on a discontinuous bovine serum albumin (BSA) gradient of 10, 28 and 35% BSA ($\rho = 1.099, 1.080$ and 1.031) for 30 min at $10,000g$. The interphase (α band) 10–28% was removed and cultured for 90 min in glass Petri dishes. Non-adherent (Nad) cells were discarded and the medium was replaced. After a further 18 h in culture, Nad cells were separated by Fc receptor (FcR) rosetting. FcR-positive and FcR-negative (dendritic) cells were then separated over a discontinuous 10–31% BSA gradient. Fractionation of NSC yields a recovery of 9–12% α -band cells, 0.3–0.5% 18-h Nad cells and finally 0.1–0.2% FcR-negative dendritic cells. H-Y-specific *T_c* cells were generated as described previously³ with minor modifications. Briefly, spleen cells (10^7) from *in vivo*-primed bm12 female mice were stimulated with irradiated (2,500 rad) male spleen cells (10^7), LPS blasts (10^6) or DC (10^5) in 2 ml medium for 5 days at $37^\circ C$ in humidified air with 5% CO_2 . Effector cells were tested on ^{51}Cr -labelled male and female bm12 LPS target cells (15×10^3) at various effector/target (E/T) ratios. The results shown are those obtained at E/T = 16 (mean of five experiments, s.e.m. indicated by bars). The % lysis on female bm12 LPS target cells was always $<6\%$. The supernatant of concanavalin A-stimulated rat spleen cells (final concentration 25% in culture medium) was used as a source of IL-2.

with female DC admixed with male NSC was ineffective (data not shown). From the results shown in Fig. 1 we conclude that only after priming of bm12 female mice *in vivo* with bm12 male DC are H-Y-specific *T_h* cells activated; the latter are needed for the generation of sufficient numbers of *T_c* memory cells. This is reflected in the finding that only after priming *in vivo* with male DC can the requirement for *T_h* cells *in vitro* be circumvented by the addition of IL-2 (Fig. 1).

Table 1 Effect of priming of bm12 female mice with male dendritic cells (DC) on the rejection of H-Y disparate skin grafts

Group	Mice	Immunization	Grafted with	No.	MST \pm s.d. (days)	P
1	B6 ♀	PBS	B6 ♂	14	22.5 \pm 6	
2	B6 ♀	B6 ♂ NSC	B6 ♂	8	22.6 \pm 4.0	0.10 (NS)
3	B6 ♀	B6 ♂ LPS	B6 ♂	11	11.2 \pm 4.7	0.05
4	bm12 ♀	PBS	bm12 ♂	15	>120	
5	bm12 ♀	bm12 ♂ NSC	bm12 ♂	15	>120	>0.20 (NS)
6	bm12 ♀	bm12 ♂ DC	bm12 ♂	8	21.9 \pm 2.1	<0.001

Mice were either unprimed or primed i.p. 3 weeks before skin grafting. All animals were also grafted with syngeneic female grafts which all survived indefinitely (>120 days). MST, mean survival time. P values were measured using the χ^2 test in comparison with phosphate-buffered saline (PBS) controls (groups 1 and 4 respectively for B6 and bm12). NS, not significant. LPS, LPS-stimulated lymphoblasts.

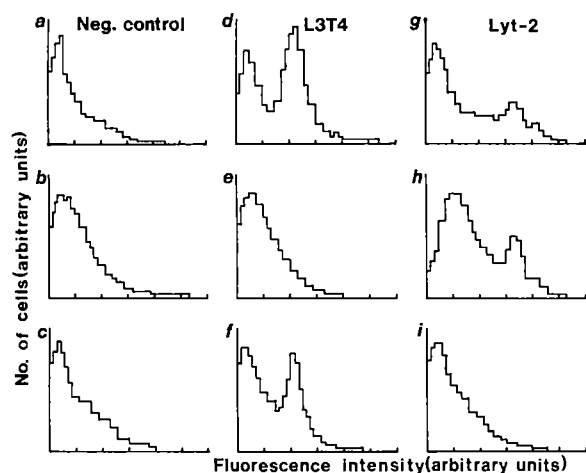


Fig. 2 Flow microfluorimetric analysis of untreated (*a, d, g*) and anti-L3T4+complement (*b, e, h*) or anti-Lyt2+complement (*c, f, i*)-treated bm12 responder cell populations (see Fig. 3 for functional analysis). Linear fluorescence histograms are expressed in arbitrary units.

Methods. After passage through nylon wool, bm12 female responder spleen cells ($2 \times 10^7 \text{ ml}^{-1}$) were incubated in Iscove's medium, 0.5% BSA, with anti-Lyt 2.2 (a mouse monoclonal antibody, 1:1,000 dilution of ascites fluid; NEN) or anti-L3T4 (a rat monoclonal antibody, 1:40 dilution of hybridoma culture supernatant SN 172-4; given by Dr H. R. MacDonald). After incubation for 45 min at room temperature, low-tox-M rabbit complement (Cedarlane) was added to a 1:10 final dilution. After incubation for 45 min at 37 °C, the cells were washed and the complement treatment was repeated. Treated cells were passed over lympholyte-M (Cedarlane; density = 1.0875). Viability was >90% and cell recovery after passage through nylon wool and treatment with L3T4 or Lyt 2+C' was $2 \pm 0.7\%$ and $3.1 \pm 0.9\%$, respectively (mean \pm s.d. of five experiments). For flow microfluorimetric analysis, aliquots of 5×10^5 untreated L3T4-negative or Lyt 2-negative responder cells in 100 μl were incubated at 4 °C with the following rat monoclonal antibodies: *a-c*, no antibody; *d-f*, anti-L3T4 (1:50 dilution of hybridoma culture supernatant H129-19 (ref. 30), provided by M. Pierres); *g-i*, anti-Lyt 2 (1:4 dilution of hybridoma culture supernatant 53-6.7 (ref. 31), provided by J. Ledbetter). After 30 min the cells were washed and $25 \mu\text{g ml}^{-1}$ of fluorescein isothiocyanate-conjugated rabbit anti-rat immunoglobulin (KR 116F, CLB) was added for an additional 30 min at 4 °C. Control samples were stained with second-step reagent only. Cells were washed three times and all samples were passed through a flow cytometer (FACS IV; Becton and Dickinson).

In further experiments we attempted to analyse whether, during *in vitro* restimulation, bm12 mutant DC stimulate L3T4⁺ T_h cells, or whether they are possibly more efficient in stimulating Lyt 2⁺ precursors of H-Y-specific helper-independent cytolytic cells. Therefore, we treated responder spleen cells with either anti-L3T4 or anti-Lyt 2 monoclonal antibody plus complement (C'), then cultured these cells with male DC as stimulator cells. Flow cytometric analysis of aliquots of the different responder cell populations (Fig. 2) showed that almost no L3T4⁺ (Fig. 2*e*) or Lyt 2⁺ cells (Fig. 2*i*) were detected after treatment with antibody and complement compared with untreated cells (Fig. 2*a, d, g*). Treatment with anti-Lyt 2 plus C', as expected, resulted in complete abrogation of the H-Y-specific T_c response (Fig. 3, population 4). In addition, after removal of L3T4⁺ T_h cells from the responder cell population, no significant H-Y-specific T_c response was generated (Fig. 3, population 3). These effects were due to the specific elimination of either L3T4⁺ or Lyt 2⁺ cells, as the response could be completely restored by mixing these two treated populations (Fig. 3, population 5). We conclude that bm12 male DC are more efficient in stimulating T_h cells than T_c precursors.

It is unknown why DC can so efficiently activate T_h cells, resulting in the abolition of the H-Y-specific immune response defect in bm12 mice. A direct relationship has been postulated

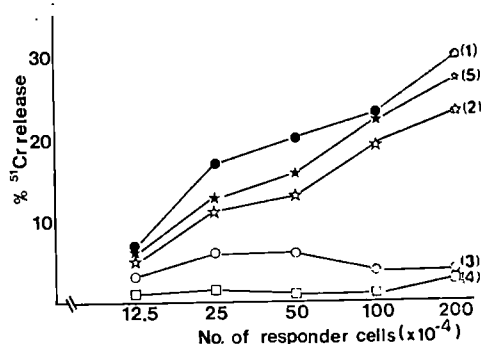


Fig. 3 Effect of responder cell treatment with anti-L3T4 or anti-Lyt 2 monoclonal antibodies plus complement on the generation of an H-Y-specific T_c response of bm12 I-A mutant mice after priming and restimulation with bm12 male DC. Treatment of responder cells: population (1) untreated; (2) medium plus complement alone; (3) anti-L3T4 plus complement; (4) anti-Lyt 2 plus complement; (5) a mixture of responder cells treated with anti-L3T4+complement (1×10^6) or anti-Lyt 2+complement (1×10^6), respectively [populations (3) and (4)].

Methods. The different cell populations were isolated as described in Fig. 2 legend. Responder cells (2×10^6) from bm12 female mice primed *in vivo* with bm12 male DC (10^5) were cultured with irradiated (2,500 rad) bm12 male DC stimulator cells (10^5) in 200 μl of culture medium in 96-well round-bottomed microtitre plates (Flow Laboratories). After 5 days of incubation in humidified air with 5% CO₂ at 37 °C, various dilutions of the effector cells were tested on ⁵¹Cr-labelled male and female bm12 LPS target cells (5×10^3). The % lysis on female target cells was always <5%.

between APC function and the number of APC surface Ia molecules¹⁹⁻²²; our results could be in agreement with this notion as bm12 splenocytes are reported to express only 25-50% as much class II antigen as B6 splenocytes^{4,23,24}, and because DC express a large number of class II molecules^{25,26}. On the other hand, DC can be expected to overcome nonresponsiveness also in cases where no quantitative differences in class II molecules between responder (R) and nonresponder (NR) strains exist (C.J.P.B., unpublished results). In general, these effects of DC can be ascribed to (1) high expression of class II molecules and (2) qualitative properties of DC as cells specialized for the APC function. Usually, T cells from F₁ hybrids between R and NR strains respond only to R-type and not to NR-type APC^{27,28}; this finding neither proves nor negates defective antigen presentation by APC as the cause of nonresponsiveness (discussed by Nagy *et al.*²⁹). We previously reported, however, that (B6 \times bm12)F₁ T cells generated an H-Y-specific T_c response to either R-type B6 or NR-type bm12 male APC³, indicating that in the bm12 mouse nonresponsiveness is not caused by defective antigen presentation but by a T-cell repertoire defect³. Despite the fact that the T_c response defect of bm12 mice can be overcome by immunization with DC, the repertoire defect for stimulation by NSC is still present: after priming with DC and restimulation with NSC no H-Y-specific T_c response occurs, unless IL-2 is added to the culture medium. Indeed, the H-Y-specific T-cell repertoire elicited by the DC immunizations is a clearly different one, because it requires not only priming but also restimulation with male DC. This DC-unique repertoire probably reflects the presence of T_h cells capable of responding to high-density I-A molecules and H-Y antigen on the surface of DC. The arousal of this dormant repertoire by immunization with DC has clear implications for states of autoimmunity, immunodeficiency and transplantation immunity.

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Phosphatidylinositol is the membrane-anchoring domain of the Thy-1 glycoprotein

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Glycoproteins exposed on cell surfaces are commonly anchored in the membrane via hydrophobic peptide domains which penetrate the lipid bilayer¹. However, it has recently been appreciated that there are exceptions to this generalization and certain cell-surface proteins appear to be anchored via a specific association with phosphatidylinositol²⁻⁷. Thy-1 glycoprotein may also be attached to cell membranes by a non-protein hydrophobic domain located at the C-terminus and although the chemical nature of this moiety has not been determined, it was postulated that it might be a lipid⁸. On the other hand, amino-acid sequences predicted from nucleotide sequence analyses suggest that a C-terminal hydrophobic peptide segment not found in the purified, detergent-solubilized Thy-1 glycoprotein may be responsible for attachment⁹⁻¹¹. We report here that a highly purified phospholipase C specific for phosphatidylinositol selectively released Thy-1 from viable normal or malignant T lymphocytes. This result supports the proposed lipid nature of the Thy-1 anchoring domain⁸ and further suggests that this lipid is, or is closely related to, phosphatidylinositol.

EL-4 murine thymoma cells were incubated with phosphatidylinositol-specific phospholipase C (PI-PLC) purified from *Staphylococcus aureus* culture supernatants¹². The cells were washed in media, stained with fluorescently labelled anti-Thy-1 antibodies and analysed by flow cytometry. Treatment with PI-PLC produced substantial decreases in cell-surface Thy-1, as indicated by a downward shift in relative cell fluorescence (Fig. 1a). The reduction in Thy-1 density after PI-PLC treatment

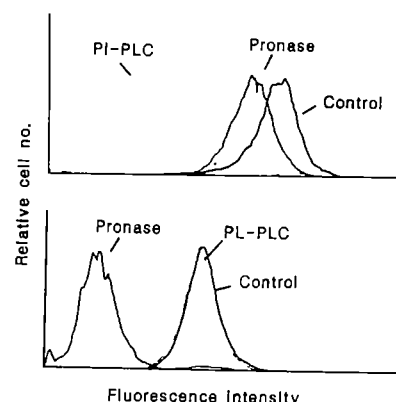


Fig. 1 Cells were incubated with PI-PLC or pronase and analysed for surface antigen as described in Table 1 legend. For clarity, the location of the unstained controls is not shown, but data from this and other experiments normalized with respect to unstained controls are shown in Table 1. *a*, Thy-1 antigen on EL4 cells; *b*, 14.8 antigen on 70Z/3 cells.

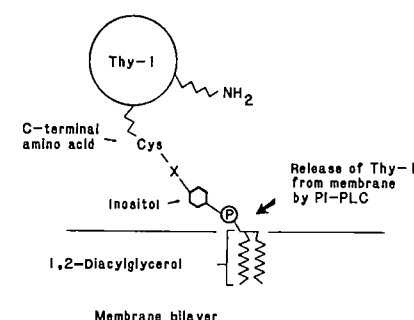


Fig. 2 A hypothetical model for Thy-1 membrane attachment. As no hydrophobic peptide domains are present in mature Thy-1 and it is released from the membrane by PI-PLC, the acyl chains of phosphatidylinositol are proposed to be entirely responsible for membrane anchoring as a result of hydrophobic interactions within the lipid bilayer. The Thy-1 polypeptide is covalently attached at the C-terminal amino-acid residue (cysteine) to the inositol ring of phosphatidylinositol via a structure (X) which at present is unidentified but appears to contain ethanolamine, glucosamine and galactosamine⁸. Similarly, the nature of the X-phosphatidylinositol linkage is unknown but probably involves one of the five free hydroxyl groups on the inositol ring.

typically amounted to a 50% reduction in logarithmically expressed fluorescence intensity and, in some experiments, a proportion of the cells was completely denuded of antigen. The effect of PI-PLC on Thy-1 was not restricted to tumour cells, as a reduction in surface Thy-1 fluorescent labelling was also observed when normal thymocytes prepared from mouse thymus were treated with PI-PLC (Table 1). By contrast, treatment of the cells with pronase (at a concentration 100 times greater than that of PI-PLC) produced a relatively small decrease in the amount of cell fluorescence (Fig. 1a, Table 1). This result indicates that Thy-1 is relatively insensitive to proteolysis, as suggested by a previous study¹³, and that loss of Thy-1 from the cell surface as a result of PI-PLC treatment is probably not due to a minor proteolytic contaminant of the highly purified PI-PLC. This view was further strengthened by the observation that other lymphocyte surface proteins such as the 14.8 antigen (on 70Z/3 cells) and the transferrin receptor (on EL4 cells) were not removed by PI-PLC even though these proteins are highly sensitive to pronase (Fig. 1b, Table 1).

The transferrin receptor has previously been shown to contain covalently bound fatty acid but it is not known whether this is involved in membrane anchoring¹⁴. It should be emphasized that PI-PLC purified by the procedure used here has previously been shown to have no detectable proteolytic activity against a

Table 1 Effect of phosphatidylinositol-specific phospholipase C on lymphocyte surface antigens

Antigen	Cells	Treatment	Fluorescence intensity (% of control)
Thy-1	EL4	PI-PLC (<i>S. aureus</i>)	51.4 ± 12.6 (7)
		Pronase	83.1 ± 10.7 (4)
		PLC (<i>B. cereus</i>)	97.6 (1)
		PLC (<i>C. perfringens</i>)	98.5 (1)
Thy-1	Mouse thymocytes	PI-PLC (<i>S. aureus</i>)	58.0 (2)
		Pronase	70.0 (1)
		PLC (<i>B. cereus</i>)	94.9 (1)
		PLC (<i>C. perfringens</i>)	96.4 (1)
Transferrin receptor	EL4	PI-PLC (<i>S. aureus</i>)	101.7 ± 31.5 (3)
		Pronase	0.8 (2)
14.8	7OZ/3	PI-PLC (<i>S. aureus</i>)	103.6 ± 4.2 (3)
		Pronase	26.9 (2)

Cells (10^6 ml^{-1}) were incubated for 60 min at 37°C in RPMI 1640/HEPES medium containing bovine serum albumin (2 mg ml^{-1}), DNase I ($200 \mu\text{g ml}^{-1}$; Calbiochem) and 2-mercaptoethanol ($5 \times 10^{-5} \text{ M}$) plus the following additions as indicated above: phosphatidylinositol-specific phospholipase C (PI-PLC), $20 \mu\text{g ml}^{-1}$ (purified from *S. aureus* culture supernatants)¹²; pronase, 2 mg ml^{-1} (*Streptomyces griseus* protease; Sigma); phospholipase C (PLC), 1 U ml^{-1} (*Bacillus cereus* grade I; Boehringer Mannheim); PLC, 5 U ml^{-1} (*Clostridium perfringens* type XII; Sigma). With the last two treatments, similar results were obtained at 10- and 100-fold lower concentrations (data not shown). The cells were centrifuged, resuspended in fresh medium and stained with fluorescently labelled antibodies. Thy-1 was demonstrated using monoclonal rat anti-mouse Thy-1.2 (clone 30-H12; Becton-Dickinson)²⁹ and monoclonal mouse anti-mouse Thy-1.2 (clone HO-13-14, from the American Type Culture Collection)³⁰; these were used as purified, directly labelled or biotin-labelled antibodies. The murine receptor for transferrin was revealed using monoclonal rat antibodies (clone R17 217) given by Dr Ian Trowbridge³¹. Monoclonal rat anti-14.8 antibody detects a macromolecule which is preferentially expressed on B-lymphocyte lineage cells³². Purified, fluorescein-labelled mouse anti-rat immunoglobulin antibodies were used as a second-step reagent as described previously³². A total of 10,000 cells per sample was evaluated with a Coulter EPICS V flow cytometer. Fluorescence was recorded as arbitrary units (channel numbers) on a logarithmic scale and median intensities determined (channel number above which 50% of cells were included). Data from individual experiments were normalized with respect to the fluorescence of unstained control samples and values are expressed as percentages ($\pm \text{s.d.}$) of the fluorescence intensities in the stained control samples. Values in parentheses are the total number of determinations in five independent experiments.

variety of substrates, to be extremely selective in the membrane components that it releases, and to hydrolyse only phosphatidylinositol and its derivatives^{3,7,15-18}. The specificity of the releasing effect of PI-PLC was further demonstrated by the observation that treatment of EL4 cells or thymocytes prepared from normal mouse thymus with two other phospholipases C which are unable to hydrolyse phosphatidylinositol^{19,20} did not have any effect on the amount of Thy-1 on the cell surface (Table 1). Thus, release of Thy-1 by PI-PLC is unlikely to be due to a physical effect resulting from perturbation of membrane structure (for example, microvesiculation) since degradation of cell-surface phospholipids using nonspecific phospholipases C would produce at least as much 1,2-diacylglycerol as the PI-PLC. The observation that the transferrin receptor and 14.8 antigen are not released (Fig. 1b, Table 1) would also argue against such a mechanism. In this regard, note that all other proteins released by PI-PLC have clearly been shown to be soluble, hydrophilic and of low relative molecular mass^{2-5,16}.

It has been suggested that the cell-surface proteins alkaline phosphatase, 5'-nucleotidase, acetylcholinesterase and the variant surface glycoprotein (VSG) of *Trypanosoma brucei*, are anchored to membranes by covalent attachment to phosphatidylinositol^{2,4-7,21}. The hydrophobic membrane-anchoring domains of the latter two proteins, which are located at the

C-terminus, have several unusual structural features. They contain ethanolamine, glucosamine and phosphatidylinositol (or inositol) but no hydrophobic amino acids^{6,7,21,22}, which suggests that the hydrophobic character and membrane attachment function in these proteins are due entirely to the acyl chains of the phosphatidylinositol. Some of these structural features of the C-terminal domain have also been observed in the Thy-1 antigen⁸. The data presented above clearly demonstrate that a substantial proportion of the Thy-1 expressed on the surface of thymocytes is selectively released by treatment with PI-PLC from *S. aureus*, and support the suggestion of Williams and co-workers⁸ that a covalently attached lipid, probably phosphatidylinositol (Fig. 2), is responsible for membrane attachment.

The role of lipid in the anchoring of Thy-1 to the membrane was recently challenged by the observation that the primary messenger RNA translation product of the *thy-1* gene, deduced from complementary DNA and genomic sequences⁹⁻¹¹ contains an extra sequence of 31 amino acids at the C-terminus which is not found in the mature protein⁸. Multiple genes, tissue/species differences, post-transcriptional mRNA processing, or post-translational protein processing do not appear to be responsible for this discrepancy^{10,11}. As this extra C-terminal sequence is extremely hydrophobic, Silver and co-workers suggested that it, instead of a lipid, is responsible for membrane attachment⁹⁻¹¹. However, the data presented here directly challenge this view as most of the surface Thy-1 on lymphocytes was released by a PI-PLC that does not cleave peptide bonds¹⁵⁻¹⁷.

Although Silver and co-workers found no evidence for post-translational proteolytic processing¹⁰, we propose that the discrepancy between the cDNA and amino-acid sequences arises as a result of the removal of the 31-amino-acid hydrophobic sequence from the C-terminus before further modification by addition of the phosphatidylinositol-containing structure. It is important to note that this same sequence of events has already been proposed for the biosynthesis and post-translational modification of the VSG of *T. brucei*^{7,23,24}. It is possible that multiple forms of Thy-1 exist and our studies, which are concerned with only those molecules exposed at the murine lymphocyte surface, do not preclude such a possibility. However, the suggestion that artefactual losses of the hydrophobic C-terminal peptide, during purification and sequence analysis, underlie the discrepancy⁹⁻¹¹ seems unlikely.

These findings beg the question of the function of this unique form of membrane attachment for particular cell-surface proteins. In the case of the VSG of trypanosomes, a specific endogenous phospholipase C has been found⁶ and it is believed that this may be important for loss of surface antigen during differentiation^{23,24}. Thy-1 is a cell-surface glycoprotein which has been well characterized with respect to distribution in animals and man, appearance in ontogeny, and amino-acid sequence²⁵. Although the function of Thy-1 is unknown, it is thought to be a member of an immunoglobulin-like family of cell-surface molecules which are involved in recognition processes^{25,26}. It is not clear whether circumstances exist in which Thy-1 is released from neuronal and lymphoid cell surfaces. However, as phosphoinositide-specific phospholipases C, which are widely distributed in mammalian tissues, are believed to be activated during cell stimulation²⁷, this possibility should be considered.

Since submission of this paper, we learned of more detailed analyses of the hydrophobic C-terminal domain of Thy-1 (ref. 28) which reveal the presence of inositol, phosphate, glycerol and fatty acid. This result strongly supports the argument presented here that phosphatidylinositol is the membrane-anchoring domain of Thy-1.

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Thrombospondin binds falciparum malaria parasitized erythrocytes and may mediate cytoadherence

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Plasmodium falciparum infected erythrocytes containing mature trophozoites and schizonts sequester along venular endothelium¹ and are not in the peripheral circulation of patients with malaria. Knobs appear on infected erythrocytes and are the points of attachment to endothelium². Sequestration may protect the parasite from splenic destruction³ and may play a role in the pathogenesis of cerebral malaria⁴. Correlates of sequestration have been developed *in vitro* using cultured human endothelium⁵ and an amelanotic melanoma cell line⁶. Knobless strains (K-) of *P. falciparum* fail to sequester *in vivo* and to bind to cells *in vitro*. We now present evidence that the receptor for cytoadherence is the glycoprotein, thrombospondin. *Aotus* monkey or human erythrocytes containing knobby (K+) but not *Aotus* erythrocytes containing knobless strains of *P. falciparum* bind to immobilized thrombospondin. Neither binds to the adhesive proteins laminin, fibronectin, factor VIII/von Willebrand factor or vitronectin. Both soluble thrombospondin and anti-thrombospondin antibodies inhibit binding of parasitized *Aotus* erythrocytes to immobilized thrombospondin and to melanoma cells which secrete thrombospondin.

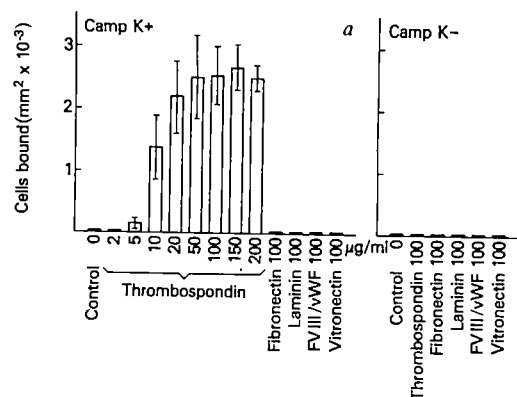


Fig. 1 Binding of *P. falciparum* parasitized erythrocytes to adhesive proteins adsorbed on plastic. *a*, K+ strain; *b*, K- strain.

Methods. Knobby (K+) and knobless (K-) variants of Malayan Camp strain *P. falciparum*²² were maintained in Columbian night monkeys (*Aotus trivirgatus griseimembra*)²⁶. Blood with 20-40% parasitaemia was cryopreserved as rings²⁷. The rings were thawed, cultured for 20-30 h to mature trophozoites and schizonts^{25,28}, washed twice in RPMI 1640, and resuspended to a 1.5-2.0% haematocrit in RPMI 1640. Viable platelets and leukocytes do not survive the cryopreservation and were not seen in the cultures. Calcium-replete human platelet TSP (isolated as described in ref. 20), human plasma fibronectin (Collaborative Research, Inc.), mouse laminin¹⁰, human factor VIII/von Willebrand factor (FVIII/vWF)²⁹, and human plasma vitronectin¹¹ in phosphate-buffered saline pH 7.4, containing 1 mM CaCl₂, were adsorbed onto bacteriological plastic dishes (Falcon 1007) by incubation in a humidified atmosphere for 3 h at 25 °C or overnight at 4 °C. These conditions result in efficient adsorption of the proteins based on immunoreactivity of adsorbed TSP¹⁴ and promotion of cell adhesion by adsorbed fibronectin, laminin and vitronectin^{9,11,30}. Control disks were incubated with phosphate-buffered saline alone. A circular area 3 mm in diameter coated with protein could be reproducibly obtained using a 10-µl drop of protein solution. After incubation, the protein solution was aspirated and the plate was immediately immersed into 50 mM Tris-buffered saline pH 7.8, containing 1% bovine serum albumin, 5 mM CaCl₂ and 0.1 mM phenylmethylsulphonyl fluoride to minimize nonspecific binding. After 30-60 min at 25 °C, the plate was rinsed in RPMI 1640 and overlaid with a suspension of parasitized erythrocytes. The plates were incubated at 37 °C for 60 min with gentle agitation every 15 min, washed by dipping in RPMI 1640, fixed for 2 h in phosphate-buffered saline, pH 7.4, containing 1% glutaraldehyde, and stained with 1% Giemsa. Results are presented as the mean \pm 1 s.d. ($n=4$).

Several adhesive glycoproteins are associated with endothelial cells or are present in blood, including thrombospondin (thrombin-sensitive protein⁷, glycoprotein G⁸, TSP), fibronectin⁹, laminin¹⁰, vitronectin¹¹ and factor VIII/von Willebrand factor¹². Adherence of Malayan Camp strain K+ *P. falciparum* parasitized *Aotus* erythrocytes to plastic surfaces coated with each of these glycoproteins or with serum albumin was tested (Fig. 1). Parasitized erythrocytes adhered avidly to surfaces coated with TSP but not to surfaces coated with laminin, fibronectin, factor VIII/von Willebrand factor, vitronectin or serum albumin. Adherence to TSP was detectable using 5 µg ml⁻¹ TSP for coating and was maximal at 50 µg ml⁻¹ TSP. Of the cells bound to TSP, 85-95% contained parasites (Fig. 2), whereas the parasitaemia of the erythrocytes used in this assay was only 10%. Uninfected erythrocytes cultured under the same conditions used for the parasitized cells did not bind to any of the proteins tested. Binding to TSP is specific for erythrocytes containing mature trophozoites or schizonts. Ring forms, the immature parasites within erythrocytes, do not sequester *in vivo* and did not bind to TSP.

A Camp K- strain of *P. falciparum*³ was also examined for adherence to proteins adsorbed on plastic (Fig. 1*b*). K- parasitized *Aotus* erythrocytes did not bind to TSP or the other adsorbed proteins. The correlation between knobby phenotype

Table 1 Binding of erythrocytes parasitized with different strains of *Plasmodium falciparum* to thrombospondin and to C32 melanoma cells

Parasite strain	No. of parasitized erythrocytes bound:	
	Per mm ² of thrombospondin-coated plate*	Per 100 melanoma cells†
<i>Aotus</i> erythrocytes		
Camp K+	7,100 ± 240	1,960 ± 90
Camp K-	25 ± 32	5 ± 2
St Lucia K+	4,100 ± 600	1,040 ± 50
St Lucia K-	30 ± 27	7 ± 3
Human erythrocytes		
Brazil ItG2F6	600 ± 350	170 ± 90
Vietnam/Cambodia (V1)	530 ± 350	290 ± 80
Thailand (T2)	220 ± 160	70 ± 30
Kenya (K3)	210 ± 140	35 ± 10
Liberia (L)	100 ± 90	115 ± 90
Nigeria (N1)	40 ± 60	50 ± 50

Parasites of the Malayan Camp²² and St Lucia²³ strains were maintained in *Aotus* monkeys and cultured synchronously to 6–20% total parasitaemia by the methods described in Fig. 1 legend. K+ and K- strains were maintained in non-splenectomized and splenectomized monkeys, respectively³. Parasites of the Brazil (ItG2F6 (ref. 24), a clone by limit dilution of It), Vietnam/Cambodia (V1), Thailand (T2), Kenya (K3), Liberia (L) and Nigeria (N1) strains all have knobs¹³ and were cultured in O⁺ human erythrocytes with O⁺ serum²⁵, cryopreserved and thawed²⁷, and cultured asynchronously in fresh erythrocytes for 10–20 days to 2–14% total parasitaemia (0.3–6% mature trophozoites and schizonts). TSP and melanoma binding assays were done four times during this period. Binding assays were performed as in ref. 6 and in Fig. 1. Binding of unparasitized erythrocytes to TSP-coated plates was 86 ± 40 cells mm⁻².

* Mean ± s.d., n = 4.

† Mean ± s.d., n = 5.

and TSP binding was further examined using another strain of *P. falciparum* (Table 1). As was found with the Camp strain, *Aotus* erythrocytes infected with the St Lucia K+ strain, but not the St Lucia K- strain, bound avidly to TSP. Only K+ strain parasitized erythrocytes bind to melanoma⁶ and endothelial cells⁵. Thus both sequestration and binding to TSP require knobs.

Although knobs are necessary for sequestration⁶, they are not sufficient¹³. Knob-positive strains of *P. falciparum* after continuous culture *in vitro* have decreased binding to melanoma or endothelial cells¹³. The strains It, V1, T2, K3, L and N1 all have knobs¹³. We measured the binding to TSP of these K+ strains cultured in human erythrocytes (Table 1). All bound at reduced levels to both TSP and melanoma cells. The two strains that bound best to melanoma cells, ItG2F6 and V1, bound best to TSP. Thus, the knobby phenotype appears to be necessary but not sufficient for TSP adherence. These results also demonstrate that parasitized human erythrocytes bind to TSP.

Further evidence that TSP is a receptor on the melanoma surface for binding of *Aotus* parasitized erythrocytes is twofold. First, preincubation of parasitized erythrocytes with soluble thrombospondin inhibited adherence both to immobilized TSP and melanoma cells (Table 2). Using 200 µg ml⁻¹ TSP, binding to TSP was inhibited 91%, and binding to melanoma cells was inhibited 81%. In a control experiment, preincubation of parasitized erythrocytes with 200 µg ml⁻¹ laminin did not inhibit binding. Second, preincubation of the melanoma cells or TSP-coated plates with rabbit anti-TSP immunoglobulin but not non-immune rabbit immunoglobulin inhibited binding (Table 2). As was the case with TSP inhibition, the antibody was more effective in inhibiting binding to TSP than binding to melanoma cells. Similar results were obtained using the monoclonal mouse antibody to TSP, A2.5 (ref. 14) (Table 2), whereas two other mouse monoclonal anti-TSP antibodies, A6.1 and C6.7 (ref. 14), did not inhibit attachment (data not shown).

Table 2 Inhibition of parasitized erythrocyte binding to immobilized thrombospondin and melanoma cells by soluble thrombospondin and anti-thrombospondin antibodies

Inhibitor	Final concentration (µg ml ⁻¹)	% Of control binding to:	
		Immobilized thrombospondin	Melanom. cells
Soluble thrombospondin	20	ND	89
	50	65	ND
	100	18	ND
	200	9	19
Rabbit anti-thrombospondin antibody	4	105	110
	12	88	ND
	40	20	63
	125	3	32
Non-immune rabbit IgG	125	120	90
Mouse monoclonal anti-thrombospondin A2.5	50	15	51

Rabbit anti-TSP antibodies and mouse monoclonal anti-TSP antibody A2.5¹⁴ were purified on Affigel-protein A (Bio-Rad). To determine the effect of TSP on binding, Camp K+ strain parasitized *Aotus* erythrocytes with 10% parasitaemia were incubated at 2% haematocrit in RPMI 1640 containing different concentrations of purified TSP at 25 °C for 30 min and then placed over TSP-coated plates (100 µg ml⁻¹) or formalin-fixed C32 melanoma cells. The TSP binding assay was continued as in Fig. 1. The melanoma cytoadherence assay was performed as in ref. 6. To determine the effect of rabbit anti-TSP antibody and mouse monoclonal anti-TSP antibody 2.5 on binding, TSP-coated plates (100 µg ml⁻¹) and melanoma cells were incubated in different concentrations of antibodies in RPMI 1640 at 25 °C for 30 min. To this solution was added a volume of parasitized erythrocytes at 10% parasitaemia and 50% haematocrit such that the final haematocrit was 2%. The TSP binding and melanoma cytoadherence assays were continued as above. ND, not determined.

A solid-phase radioimmunoassay for TSP using rabbit anti-TSP immunoglobulin coated on 96-well microtitre plates in 0.1 M sodium carbonate, pH 9.6, and ¹²⁵I-TSP¹⁵ (10 µCi µg⁻¹, 50 ng ml⁻¹) was used to detect TSP synthesis by C32 melanoma cells. To remove immunoreactive bovine TSP, melanoma cells which had been grown in RPMI 1640 containing 10% fetal bovine serum were preincubated for 16 h in medium containing 10% heat-inactivated horse serum, which does not contain immunoreactive TSP. For determination of TSP secretion, fresh medium containing 10% horse serum was incubated with the cells for 24 h, centrifuged for 10 min at 1,000g and assayed by radioimmunoassay. Dilutions of TSP in 10% horse serum were used as standards. The melanoma cells secreted 13 µg TSP per 10⁶ cells per 24 h. Human umbilical cord endothelial cells also synthesize and secrete TSP¹⁶. Thus, both target cells for cytoadherence *in vitro*, the melanoma cells and human endothelial cells, produce TSP. Immunofluorescence analysis of bovine aortic endothelial cells¹⁷ suggested that TSP is localized both intracellularly and as a component of fibrillar extracellular matrix.

A trypsin-sensitive strain-specific plasmodial protein of relative molecular mass 260,000–285,000 (ref. 18) on the surface of K+ but not K- parasitized erythrocytes¹⁹ has been implicated as a ligand for cytoadherence. However, the parasite could also expose a host ligand for TSP binding. TSP agglutinates trypsinized glutaraldehyde-fixed erythrocytes²⁰ and sulphated glycoconjugates on the fixed erythrocyte probably mediate agglutination¹⁵. The sulphated polysaccharides heparin and fucoidan bind to TSP and inhibit fixed erythrocyte agglutination¹⁵. We have shown that TSP binds parasitized erythrocytes and that heparin and fucoidan do not inhibit this binding (D.D.R., unpublished results). Therefore, a different site on TSP is probably recognized by the parasite.

Although parasitized erythrocytes bind directly to TSP adsorbed onto plastic, TSP itself may not be the only molecule

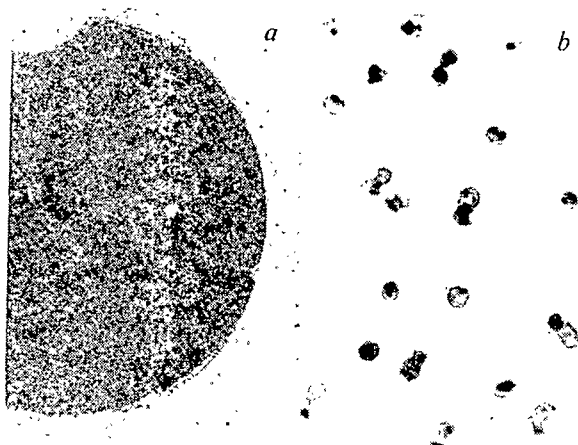


Fig. 2 Binding of *P. falciparum* parasitized erythrocytes to human platelet thrombospondin. A $100 \mu\text{g ml}^{-1}$ solution of TSP was adsorbed onto plastic dishes, and binding of Malayan Camp K+ parasitized *Aotus* erythrocytes was determined as described in Fig. 1. The dishes were fixed in 100% methanol and stained with Giemsa. The granular material bound to a 3-mm circular area coated with TSP (*a*, $\times 17$) consists of stained parasitized erythrocytes viewed at high magnification in *b* ($\times 300$).

required for cytoadherence. Since TSP is a soluble protein, receptors for TSP are probably present on the cell membrane or extracellular matrix of melanoma cells. Thus other melanoma surface molecules may be necessary in conjunction with TSP for cytoadherence. Requirement for a TSP receptor is also suggested by the finding that some cell lines which secrete TSP in culture do not bind parasites (J.A.S., manuscript in preparation).

We have established that K+ strains of *P. falciparum* in *Aotus* erythrocytes attach specifically to immobilized TSP but not to other cell adhesion molecules. K+ strains of *P. falciparum* in fresh human erythrocytes also attach to TSP. TSP is produced by both melanoma and endothelial cells¹⁶ and may be on the surface of these cells. This evidence, combined with the blocking of cytoadherence to melanoma cells by TSP and by antibodies to TSP, is consistent with the hypothesis that TSP is a melanoma and endothelial cell receptor for *P. falciparum* cytoadherence. Binding of *P. falciparum* strains to TSP correlates well with binding to melanoma cells *in vitro*. Binding to melanoma cells correlates well with binding to endothelial cells^{6,13} and with sequestration *in vivo*^{6,21}. Sequestration occurs at specific vascular sites, including cardiac venules¹. If TSP is the receptor for cytoadherence *in vivo*, endothelium in these vessels may selectively express TSP on their luminal surfaces.

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Sequence of human tissue inhibitor of metalloproteinases and its identity to erythroid-potentiating activity

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Collagen fibres form the stable architecture of connective tissues and their breakdown is a key irreversible step in many pathological conditions^{1,2}. The destruction of collagen is usually initiated by proteinases, the best known of which is the metalloproteinase collagenase (EC 3.4.24)³. Collagenase and related metalloproteinases are regulated at the level of their synthesis and secretion, through the action of specific stimuli such as hormones and cytokines, and also at the level of their extracellular activity through the action of a specific inhibitor, TIMP (tissue inhibitor of metalloproteinases)⁴⁻⁶, which irreversibly forms inactive complexes with metalloproteinases⁷. Although the mechanisms governing the production of TIMP are unknown, immunologically identical forms of this glycoprotein have been detected in a wide variety of human body fluids and cell and tissue culture media^{5,8}. We therefore suggested⁵ that under physiological conditions this ubiquitous inhibitor predominates over active metalloproteinases and that tissue destruction may arise when any perturbation of this controlling excess arises. However, further progress towards testing this theory has been hindered by a lack of knowledge about the structure of TIMP and insufficient material for studying it in model systems. Here we describe the structure of TIMP predicted from its complementary DNA, its synthesis in *Escherichia coli* and transfected animal cells, and the finding that it is identical to a protein recently reported to have erythroid-potentiating activity (EPA)⁹.

TIMP was sequenced after purification from human amniotic fluid¹⁰ and from the culture media of human fetal lung fibroblasts (American Type Culture Collection CCL153). Figure 1*a* compares these N-terminal sequences with that published for a collagenase inhibitor¹¹, with properties similar to TIMP⁸, that is produced by human skin fibroblasts. Human amniotic fluid and human fetal lung TIMP are identical for all 28 N-terminal amino acids identified. Except for a leucine (instead of lysine at position 22) the human skin fibroblast inhibitor is also identical for 23 N-terminal residues¹¹ (Fig. 1*b*). Based on this information, we constructed a single 69-base oligonucleotide probe capable of encoding the 23 N-terminal amino acids of TIMP¹² (Fig. 1*c*). The codons used were those reported to appear most frequently in human genes, except in three positions where to

Fig. 1 N-terminal amino-acid sequences of human fetal lung fibroblast and human amniotic fluid TIMP (a) and of human skin fibroblast collagenase inhibitor (b). c, The 69-base oligonucleotide probe; d, the complementary 18-mer. The numbers above the probe sequence refer to the nucleotide sequence of the cDNA (Fig. 2b). The letters above the probe sequence represent those nucleotides that are different in the cDNA.

Methods. Human fetal diploid lung cells (ATCC CCL153) purchased at passage 13 (Flow Laboratories) were maintained in Dulbecco's modified Eagle's medium (DMEM; Gibco) supplemented with 4 mM glutamine, 15% heat-inactivated fetal calf serum (FCS) and 100 IU ml^{-1} penicillin and 100 $\mu\text{g ml}^{-1}$ streptomycin. Cells were expanded into two 490- cm^2 plastic roller bottles, allowed to reach confluence in growth medium, washed with serum-free DMEM and then maintained in serum-free media for up to 6 days. Culture supernatant TIMP was measured by inhibition of rabbit skin collagenase using the collagen fibril assay¹⁰. One unit of TIMP inhibits 2 units of collagenase (2 μg of collagen degraded per min) by 50%. TIMP levels varied from <0.2 U to 5 U per 10^5 cells per 48 h, and low-producing cells were stimulated by the addition of 5 ng ml^{-1} 4 β -phorbol-12 β -myristate-13 α -acetate (PMA) to the serum-free culture medium²⁴. Culture supernatants and cells were collected after 6 days, the former being concentrated 40-fold with an Amicon concentrator using a Diaflo hollow-fibre cartridge with a M_r -10,000 cut-off. TIMP was purified from the concentrated supernatants¹⁰ and reduced and carboxymethylated by standard procedures. TIMP from human amniotic fluid was similarly purified¹⁰, reduced and carboxymethylated. The NH_2 -terminal sequence was determined by automated Edman degradation on an Applied Biosystems gas-phase sequencer. The 69-mer oligonucleotide probe was synthesized on an Applied Biosystems model 380A synthesizer by monomer addition of activated phosphoamidites to a solid support. The deprotected probe was purified by HPLC. The 18-mer was synthesized by automated solid-phase phosphotriester chemistry²⁵ and also purified by HPLC.

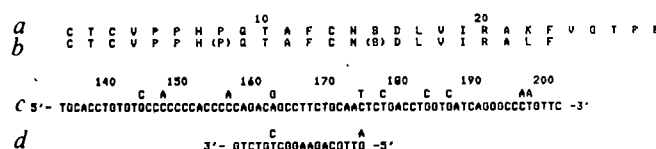


Fig. 2 a, Restriction map of human TIMP cDNA compiled from one almost full-length cDNA and the 5' end (broken line) of one additional overlapping cDNA. The open boxes represent pBR322 sequences. The arrows denote directions of dideoxy sequencing. P, *Pst*I; N, *Nco*I; T, *Tth*III-1; B, *Bst*XI; M, *Mst*II; A, *Ava*I; V, *Pvu*I; H, *Hae*II; I, *Hin*fl. b, Nucleotide sequence of human TIMP and the predicted amino-acid sequence. The numbers beneath the sequence refer to the mRNA sequence. The nucleotides at positions 27 and 706 were not identified. The numbers above the sequence refer to the amino-acid sequence; negative numbers refer to the signal sequence. Potential glycosylation sites are underlined.

Methods. mRNA was isolated from washed CCL153 cells by the guanidinium isothiocyanate/hot phenol method²⁶, followed by oligo(dT)-cellulose (Collaborative Research) column chromatography²⁷. Standard procedures were used to synthesize cDNA²⁸ and a library of 20,000 colonies was established by C-tailing the cDNA into G-tailed²⁹, *Pst*I-cut pBR322 and transforming the annealed DNA into *E. coli* DH1 (ref. 30). The colonies were transferred to nitrocellulose filters and screened with the 69-mer labelled at the 5' end to a specific activity of 2×10^7 c.p.m. μg^{-1} . Hybridization was in 0.9 M NaCl, 0.09 M Tris-HCl pH 7.4, 0.006 M EDTA, 0.5% Nonidet P-40, 2 \times Denhardt's solution, 0.2% SDS for 16 h at 42 $^{\circ}\text{C}$ with a probe concentration of 25 ng ml^{-1} . High-stringency washing of the filters (0.5 \times SSC, 0.1% SDS for 15 min at 50 $^{\circ}\text{C}$) was followed by autoradiography for 20 h with an intensifying screen. Ten putatively positive clones were identified, six of which gave positive signals after a second screening. Plasmid DNA was isolated and used as a template for T-tracking using the 18-mer as a primer^{31,32}. Five of the six clones were found to have related sequences over a region of at least 70 nucleotides and restriction mapping was used to confirm the relatedness of these clones. The complete nucleotide sequence of clone 2 (the longest clone), with some additional 5' sequence from clone 3, was determined by the dideoxy method³² using either the 18-mer to prime sequencing directly on plasmid DNA³¹ or after subcloning restriction fragments into M13 tg130 or tg131 (Amersham) and transformation into *E. coli* JM101 (ref. 33).

have included such codons would have incorporated the rarely observed dinucleotide, CpG (refs 13, 14). In addition, an 18-mer complementary to nucleotides 25–42 of the long probe was also constructed (Fig. 1d).

The 69-mer was used to identify positive colonies from a cDNA library of 20,000 clones derived from human fetal lung fibroblast messenger RNA. Restriction mapping (Fig. 2a) and partial DNA sequencing using the 18-mer as a primer revealed that five positive colonies harboured plasmids with related cDNA inserts. These were shown to be putative TIMP cDNAs by end-labelling the 18-mer and using it to prime dideoxy sequencing of the longest clone. The complete nucleotide sequence compiled from this and one other clone is shown in Fig. 2b. Examination of the sequence reveals that there is an open reading frame coding for a protein of 207 amino acids.

Evidence that the sequence encodes TIMP comes from the observation that nucleotides 133–216 encode the 28 N-terminal amino acids of human amniotic fluid and fetal lung fibroblast TIMP, including the lysine at position 22 identified by amino-acid sequencing (Fig. 1a). As the methionine encoded by nucleotides 64–66 is the only one in any reading frame upstream from the N-terminal amino acids, it is assumed that amino acids –23 to –1 (many of which are hydrophobic) represent a signal sequence that is cleaved off to liberate mature protein¹⁵. Unprocessed TIMP with the signal sequence is therefore predicted to have a relative molecular mass (M_r) of 23,144; this approximates to the size of TIMP core protein (M_r , 22,000) obtained after immunoprecipitation of the *in vitro* translation products of human fetal lung fibroblast mRNA¹⁰. Furthermore, the predicted M_r of unglycosylated mature TIMP is 20,685, which is consistent

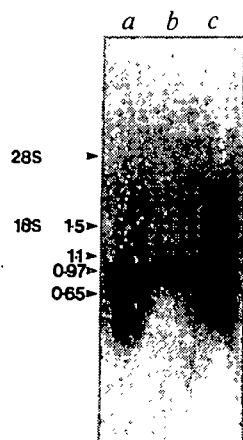


Fig. 3 Northern blot of CCL153 and HL60 mRNA probed with TIMP cDNA. *a*, CCL153 cell mRNA; *b*, mRNA from HL60 cells grown without stimulation; *c*, mRNA from HL60 cells stimulated with phorbol esters and lipopolysaccharide (LPS).

Methods. The CCL153 cells were grown as described in Fig. 1 legend. The HL60 cells were grown in a similar medium and stimulated to differentiate¹⁹ by incubation with 3×10^{-6} M PMA for 2 h, then washed and incubated for 10 h in the presence of $1 \mu\text{g ml}^{-1}$ LPS (Sigma; L-2880). mRNA was isolated as described in Fig. 2 legend and $5 \mu\text{g}$ was electrophoresed in a 1.25% agarose gel containing 8% formaldehyde, transferred to nitrocellulose and hybridized in $5 \times \text{SSC}$, $5 \times$ Denhardt's solution, 50% formamide, $300 \mu\text{g ml}^{-1}$ denatured salmon sperm DNA, 0.1% SDS, 50 mM Na_2HPO_4 pH 6.5 for 16 h at 42°C with 30 ng ml^{-1} of purified *Pst*I fragments of the cDNA shown in Fig. 2, labelled by nick-translation to a specific activity of 2×10^8 c.p.m. μg^{-1} . The filters were washed in $0.1 \times \text{SSC}$, 0.1% SDS at 60°C for 30 min and autoradiographed for 8 h. RNA sizes were estimated by running 18S and 28S RNA and ^{32}P -labelled denatured λ DNA digested with *Cla*I in parallel.

with the finding that TIMP immunoprecipitated from culture supernatants of CCL53 cells grown in the presence of tunicamycin had a M_r of 20,000 (20K)¹⁶. As tunicamycin inhibits the assembly of dolichol-linked oligosaccharides for the formation of asparagine-linked sugar chains on glycoproteins¹⁷, it is likely that most, if not all, the oligosaccharide chains on TIMP are *N*-linked. Two potential *N*-glycosylation sites are indicated in Fig. 2b.

The poly(A) tract preceded by the polyadenylation signal AATAAA at the 3' end of the cDNA indicates that the 3' end of the mRNA is represented¹⁸. Furthermore, a single band of ~800 nucleotides was observed after Northern blotting of mRNA from human fetal lung fibroblasts with TIMP cDNA, indicating that the cDNA was very close to full length (Fig. 3a). A similar-sized single band was also observed after probing mRNA from HL60 cells which had been stimulated with phorbol esters to achieve monocyte differentiation¹⁹ (Fig. 3b, c).

To confirm that the cDNA described above encodes TIMP, part of the cDNA without the signal sequence but with an additional N-terminal methionine was inserted into the *E. coli* expression vector pMG196 (E.M.W., G. O. Humphreys and G. T. Yarranton, in preparation) to produce pMG454 (Fig. 4a). In this plasmid the *trp* promoter is used to drive transcription of the inserted TIMP cDNA. pMG454 exists stably in *E. coli* at low copy number in the absence of selection but on induction increases in copy number to 100–200 per chromosome. As shown in Fig. 4c, after induction of *E. coli* cells harbouring pMG454, we observed a protein of M_r 20,000, the expected size for unglycosylated TIMP. Note that β -lactamase (which is also encoded by pMG454) was also specifically induced. By Western blotting it was shown that the 20 K protein reacts with polyclonal antibodies directed against TIMP purified from human amniotic fluid²⁰ (Fig. 4d, lane 2). As with several other recombinant proteins expressed in *E. coli*²¹, TIMP was found to be mostly in an insoluble form in inclusion bodies (data not shown).

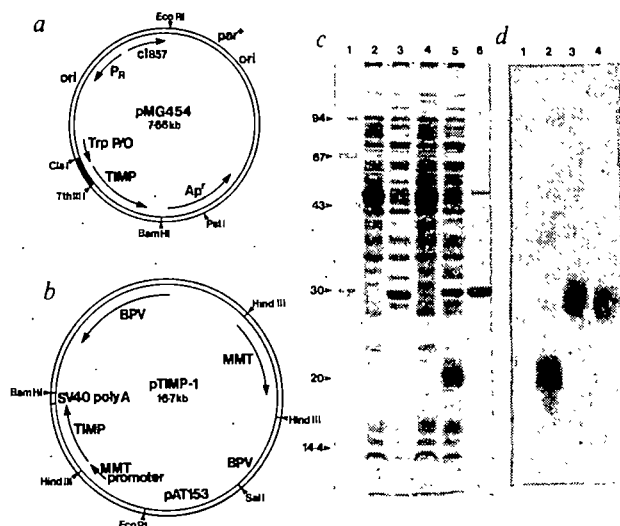


Fig. 4 *a*, *b*, Restriction maps (not to scale) of the *E. coli* expression vector pMG454 and the mammalian cell expression vector pTIMP-1, respectively. In pMG454 the solid box represents the 61-base pair (bp) linker. *c*, A 12% SDS-polyacrylamide gel showing Coomassie blue-stained proteins made in *E. coli* E103S transformed with pMG454. Lane 1, marker proteins; lane 2, non-induced control cells harbouring the expression vector with no TIMP cDNA insert; lane 3, induced control cells; lane 4, non-induced E103S cells harbouring pMG454; lane 5, as lane 4 but induced; lane 6, purified *E. coli* β -lactamase. *d*, Autoradiograph obtained after Western blotting with the TIMP antibody. Lanes 1 and 2, as lanes 4 and 5 in *c*; lane 3, purified human amniotic fluid TIMP; lane 4, culture supernatant proteins from a C127 cell line transfected with pTIMP-1.

Methods. For insertion into both expression vectors, the *Hinf*I site adjacent to the TIMP stop codon (Fig. 2) was converted with a linker into a *Bam*HI site. For expression in *E. coli* the 5' end of the cDNA was reconstructed using a 61-bp linker to provide an upstream *Cla*I site followed by an ATG start codon preceding the cysteine codon at the N-terminus of mature TIMP. The resulting TIMP-coding fragment was ligated into the dual-origin vector pMG196 and transformed into *E. coli* E103S (a proteinase-deficient K-12 derivative). Cultures of E103S containing the resulting plasmid (pMG454) were grown at 30°C in L-broth to an A_{600} of 0.4, then shifted to 42°C by shaking at 65°C . On reaching 42°C they were transferred to a 37°C water bath and grown for a further 5 h, after which appropriately sized aliquots were taken and analysed by SDS-gel electrophoresis³⁴ and Western blotting³⁵. For expression in mammalian cells the *Nco*I site adjacent to the ATG start codon was converted with a linker into a *Hind*III site. The entire TIMP coding sequence was then inserted downstream from the *MMT* promoter in the BPV-based vector shown above (P.E.S., M. M. Bendig and C. C. Hentschel, manuscript in preparation). After transfection into C127 cells³⁶ by calcium phosphate co-precipitation³⁷, CdCl_2 (20 μM)- and ZnCl_2 (20 μM)-resistant foci were selected. Their ability to secrete TIMP was measured after 16 h of growth in serum-free media by a competitive RIA based on immobilized specific antibodies to TIMP²⁰, Western blotting³⁵, and a collagen fibril assay as described for CCL153 cells (Fig. 1).

The cDNA was also inserted into a vector designed for expression in mammalian cells (Fig. 4b). The coding sequence of pre-TIMP was inserted between the murine metallothionein (*MMT*) promoter and the simian virus 40 early transcript polyadenylation signal in a bovine papillomavirus (BPV)-based vector²². This plasmid (pTIMP-1) also has a functional *MMT* gene which confers heavy-metal resistance on mammary fibroblast tumour cells (C127 cells) harbouring it. Calcium phosphate co-precipitation was used to introduce pTIMP-1 DNA into these cells and nine resistant foci were selected for further study. Radioimmunoassays (RIAs) for TIMP were performed on culture supernatants after 16 h in serum-free medium. The polyclonal antibodies directed against purified human amniotic fluid TIMP, which are known not to cross-react with bovine or murine

TIMP²⁰, detected a 200-fold increase of TIMP in some of the pTIMP-1-transfected cell lines compared with control cells transfected with the plasmid without inserted cDNA. Highest yields of up to 1.5–2.0 $\mu\text{g ml}^{-1}$ TIMP were obtained. In addition, Western blotting indicated that the TIMP secreted by the transfected C127 cells had a M_r identical to authentic TIMP purified from human amniotic fluid, suggesting that in these cells the recombinant TIMP is correctly processed and glycosylated (Fig. 4d, lane 4). To determine whether the immunoreactive TIMP made in the C127 cells was biologically active, the culture supernatants were tested for their ability to inhibit rabbit skin collagenase using the collagen fibril assay¹⁰ (see Fig. 1 legend). No activity was detected in the supernatants of those cells shown by RIA to be making undetectable amounts of TIMP. However, 2–5 U ml^{-1} TIMP was detected in the supernatants of those cells shown in the RIA to be making in excess of 500 ng ml^{-1} TIMP, indicating that pTIMP-1 directs the synthesis of active TIMP in C127 cells. Further refinement of pTIMP-1-harboring cell lines should permit a greater yield of active TIMP.

The N-terminal sequences indicate that TIMP and the human skin fibroblast inhibitor are identical. Their overall amino-acid composition is also very similar, which is in keeping with the finding that these commonly occurring inhibitors, together with others of M_r 28,000 (28K) identified in many human tissues^{5,8}, are immunologically and functionally identical. The cDNA predicts the presence of 12 cysteine residues, which is in contrast to the larger number (24 half-cystines) reported for the skin fibroblast inhibitor¹¹ but which is consistent with the cysteine content reported for a bovine metalloproteinase inhibitor²³. Recently, Gasson *et al.*⁹ reported the N-terminal sequence of a 28K protein reported to have erythroid-potentiating activity (EPA) that was purified from medium conditioned by human T-cell lymphotropic virus type II (HTLV-II)-infected Mo T-lymphoblast cells. This N-terminal sequence and the predicted amino-acid sequence of the corresponding cDNA are identical to those reported here for TIMP. Although these data suggest that the enzyme inhibitor (TIMP) may have lymphokine activity on specific cells of the erythroid lineage, it will first be necessary to determine whether TIMP prepared by our methods has erythroid-potentiating activity. The TIMP-synthesizing *E. coli* and mammalian cell lines should also permit the purification of sufficient TIMP for a thorough study of the cellular mechanisms that normally prevent uncontrolled resorption. Furthermore, the primary structure of TIMP described here will provide a starting point for designing new compounds for controlling disease processes in which accelerated breakdown of extracellular matrices is a prominent feature.

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L-myc, a new myc-related gene amplified and expressed in human small cell lung cancer

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Altered structure and regulation of the c-myc proto-oncogene have been associated with a variety of human tumours and derivative cell lines, including Burkitt's lymphoma^{1,2}, promyelocytic leukaemia^{3,4} and small cell lung cancer (SCLC)⁵. The N-myc gene, first detected by its homology to the second exon of the c-myc gene, is amplified and/or expressed in tumours or cell lines derived from neuroblastoma^{6–8}, retinoblastoma⁹ and SCLC¹⁰. Here we describe a third myc-related gene (L-myc) cloned from SCLC DNA with homology to a small region of both the c-myc and N-myc genes. Human genomic DNA shows an EcoRI restriction fragment length polymorphism (RFLP) of L-myc defined by two alleles (10.0- and 6.6-kilobase (kb) EcoRI fragments), neither associated disproportionately with SCLC. Mouse and hamster DNAs exhibit a 12-kb EcoRI L-myc homologue, which indicates conservation of the gene in mammals. Gene mapping studies assign L-myc to human chromosome region 1p32, a location distinct from that of either c-myc^{1,11} or N-myc¹² but associated with cytogenetic abnormalities in certain human tumours¹³. This L-myc sequence is amplified 10–20-fold in four SCLC cell line DNAs and in one SCLC tumour specimen taken directly from a patient. Either the 10.0- or 6.6-kb allele can be amplified and in heterozygotes only one of the two alleles was amplified in any SCLC genome. SCLC cell lines with amplified L-myc sequences express L-myc-derived transcripts not seen in SCLC with amplified c-myc or N-myc genes. In addition, some SCLCs without amplification also express L-myc-related transcripts. Together, these findings suggest an enlarging role for myc-related genes in human lung cancer and provide evidence for the concept of a myc family of proto-oncogenes.

While examining genomic DNA from human SCLC cell lines for evidence of c-myc and N-myc amplification, we occasionally observed a number of additional EcoRI fragments, including 10.0- and 6.6-kb fragments that hybridized to either a second-exon c-myc probe¹⁴ or to the homologous region of the N-myc gene (pNb-1)⁶ (see Fig. 1a). We speculated that these EcoRI

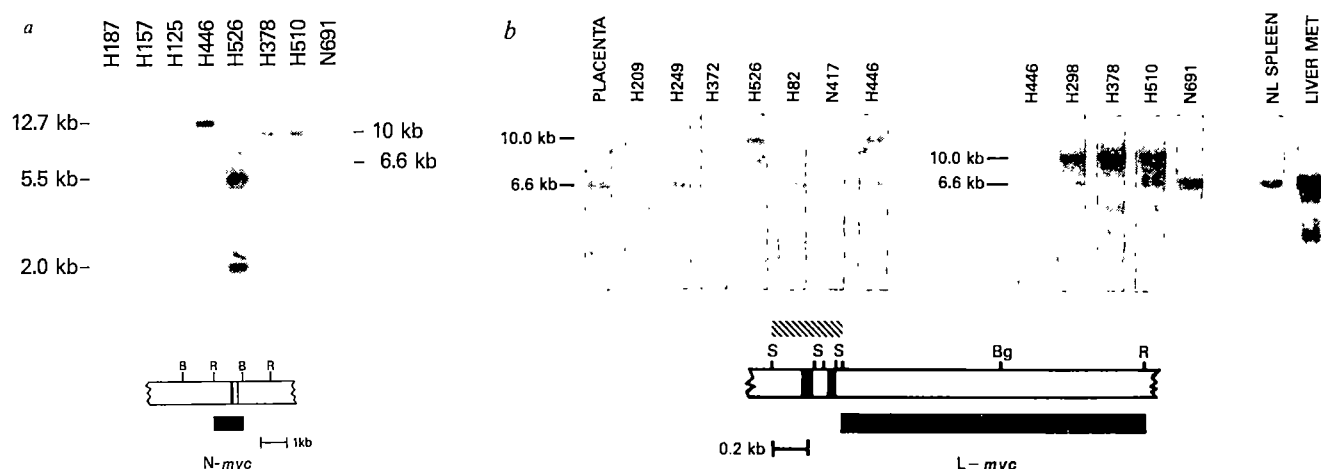


Fig. 1 *a*, Detection of novel *myc*-related sequences in *Eco*RI digests of SCLC cell line DNAs with a human *N-myc* probe. *b*, Hybridization comparison of *Eco*RI digests of SCLC cell line and patient tissue sample DNAs with a human *L-myc* probe. In *a*, the *N-myc* probe is a 1.0-kb *Eco*RI–*Bam*HI fragment (horizontal solid bar) obtained from clone pNb-1 isolated from a human neuroblastoma cell line⁶. The 12.7-kb position represents the *Eco*RI germline *c-myc* gene fragment¹ (which is seen with the *N-myc* probe when it is amplified as in H446); the 2.0-kb position, the *Eco*RI germline *N-myc* fragment⁶ seen in all DNAs in *a* and amplified in H526 (ref. 10); the 5.5-kb position, a putative *N-myc*-related gene sequence found and amplified in H526 (ref. 10); the 10.0-kb (H378 and H510) and 6.6-kb (N691) positions, the *Eco*RI *myc*-related gene fragments representing *L-myc*. In *b*, the *L-myc* probe used is the 1.8-kb *Sma*I–*Eco*RI fragment²⁷ obtained from clone pLmyc 10. The characteristic 10.0-kb *Eco*RI DNA fragment of *L-myc* was isolated by preparative gel electrophoresis²⁷ of an *Eco*RI digest of SCLC cell line H378 genomic DNA and cloned into CH4A phage²⁸. Five clones were identified using either the Nb-1 *N-myc* probe⁶ or the second-exon 1.6-kb *Sst*I–*Sst*I *c-myc* probe¹. One of these clones was subcloned into pJB327 (a derivative of pBR327)²⁹ and was termed pLmyc 10. The 10.0-kb and 6.6-kb positions represent the *Eco*RI genomic *L-myc* fragments. The degree of *L-myc* amplification was determined by cutting-out and liquid scintillation counting of the ³²P-labelled 10.0-kb and 6.6-kb *L-myc* bands from the Southern hybridization blot shown in *b*. The c.p.m. of each amplified band was divided by the average c.p.m. of a single non-amplified allele to obtain an approximate amplification value, after background c.p.m. was subtracted from both. Heavy vertical solid lines indicate regions of homology between *c-myc*, *N-myc*⁶ and *L-myc* DNA sequences; the hatched block indicates the region sequenced (see Fig. 3 legend). Restriction endonuclease sites are indicated as follows: B, *Bam*HI; R, *Eco*RI; S, *Sma*I; and Bg, *Bgl*II.

Methods. The NCI-series of cell lines that were established, characterized and grown in our laboratory^{22,23} include SCLC lines H82, H187, H209, H249, H298, H372, H378, N417, H446, H510, H526 and N691, and non-SCLC lines H125 and H157. Cell line DNA was prepared as described elsewhere²⁴, while tumour and normal tissue samples collected directly from the patient at autopsy were first pulverized in liquid nitrogen in a blender before DNA extraction. Then 12 µg of each DNA was digested with *Eco*RI, electrophoresed on a 0.8% agarose gel, denatured and transferred to nitrocellulose essentially as described by Southern²⁵. Hybridization was performed using 10% dextran sulphate²⁶ with the ³²P-labelled probe indicated on the schematic map and washed at 52 °C in 0.1 × SSC, 0.1% SDS as described previously¹.

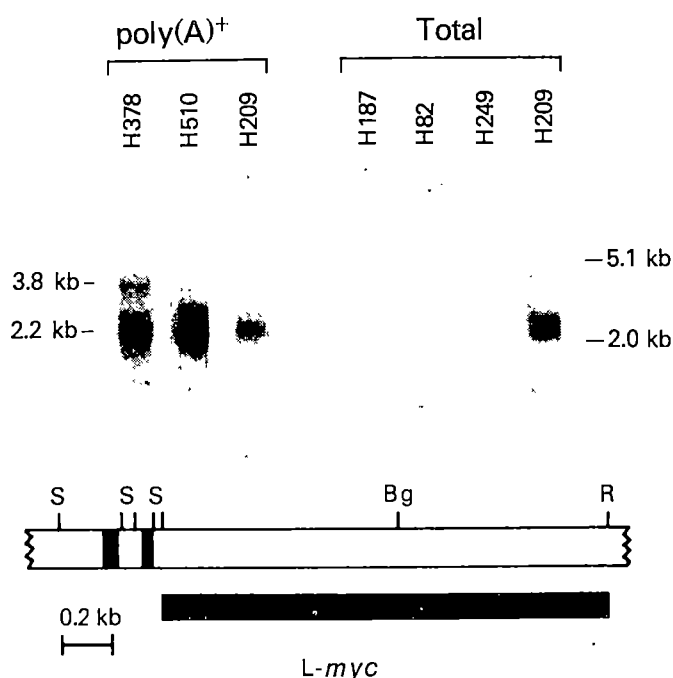


Fig. 2 Hybridization comparison of poly(A)⁺ and total SCLC cell line RNAs with a human *L-myc* probe. The 5.1-kb and 2.0-kb positions indicate 28S and 18S human ribosomal RNAs respectively.

Methods. Total cellular RNA was prepared by guanidine thiocyanate–caesium chloride gradient centrifugation³⁰. Several RNAs were further enriched by oligo(dT)–cellulose chromatography³¹. Then 10 µg of total RNA or 2 µg of poly(A)⁺ RNA was denatured and electrophoresed on a 1% agarose–formaldehyde gel³², modified by using 0.22 M formaldehyde in the gel and electrophoresing at 160 V for 4 h. Transfer, hybridization and washing were as described elsewhere³³.

myc-hybridizing fragments might represent other *myc*-related genes which were also amplified in some SCLC cell line DNAs.

To test this hypothesis, the novel 10.0-kb *Eco*RI DNA genomic fragment from human SCLC cell line NCI-H378 was cloned from *Eco*RI size-fractionated DNA and designated pLmyc 10 (see Fig. 1*b* legend). The partial pLmyc 10 restriction endonuclease map obtained (Fig. 1*b*) is markedly different from those of *c-myc*¹⁴, *N-myc*⁶ and three other *myc*-related clones described earlier¹⁵. Using restriction endonucleases, various subfragments of pLmyc 10 were subcloned, including the 1.8-kb *Sma*I–*Eco*RI fragment (see map, Fig. 1*b*). We have termed this new *myc*-related sequence *L-myc* because it was first isolated from a human lung cancer cell line.

Using the *Sma*I–*Eco*RI fragment as a probe, we then screened *Eco*RI digests of 40 SCLC cell line DNAs for *L-myc* sequences using Southern blot analysis (see Fig. 1*b* for examples). In addition to the expected 10.0-kb *Eco*RI DNA fragment, we also observed hybridization to the 6.6-kb *Eco*RI fragment in several DNAs (including human placental DNA). DNAs were identified that contained either both *Eco*RI fragments (10.0 and 6.6 kb) or only one fragment (10.0 or 6.6 kb). Since a number of other restriction endonuclease digests (*Bgl*II, *Sst*I, *Bam*HI and *Hind*III) show no pattern differences between DNAs (data not shown), these two *Eco*RI fragments identified represent an *Eco*RI restriction site polymorphism outside the *Sma*I–*Eco*RI *L-myc* probe, rather than a tumour-specific somatic rearrangement. The presence of the two alleles, however, raises the question of an association between lung cancer and the alleles, as was recently suggested for the *ras* oncogene family¹⁶. We scored 40 SCLC DNAs, 30 cell line DNAs of other tumour types including adenocarcinoma of the lung, neuroblastoma and retinoblastoma, as well as the lymphocyte DNAs of 13 normal individuals. There were no major differences in the *L-myc* allele frequencies among the three groups. Overall, 24 DNAs were 10.0-kb homozygotes, 22 DNAs were 6.6-kb homozygotes and 37 were heterozygotes. Pooling data from all tumour cell lines

Diagram illustrating the structure of the *c-myc* gene and its transcripts. The top part shows the *c-myc* gene structure with exons represented by boxes and introns by lines. The 5' and 3' ends are indicated. A scale bar indicates 500 bp. Below the gene structure, four transcripts are shown, each with its corresponding nucleotide sequence and amino acid sequence (aa.).

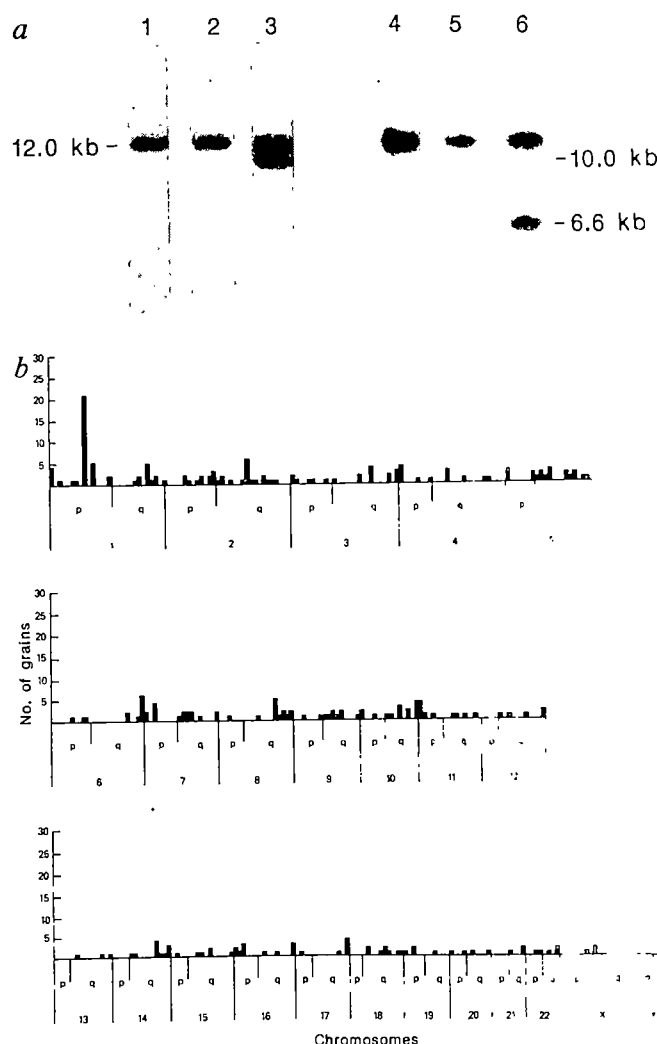
Transcripts and Sequences:

- N-myc**: C C G G G C G T G C G G C -138 bp- G C C C
- C-myc**: GAGCCCGAGGAGATATCTGGAAGAATTCGAGCTGCTGCCACCCCGCCCTGTCCCT -192 bp- ATCATCATCCAGGACTGTATGTGGACGCTCTCTCGG
- L-myc**: C G AT G C AC G G -135 bp- ACG GT C

Amino Acid Sequences (aa.):

- N-myc**: Pro - Gly - - - - - (46 aa.) Val - Leu - - - - -
- C-myc**: AlaProSerGluAspIleTrpLysPheGluLeuLeuProThrProPheLeuSerPro (44 aa.) IleIleIleGlnAspCysMetTrpSerGluPheSerAla
- L-myc**: - - - Val - Ser - Thr - - - (45 aa.) - - - ArgArg - - - - -

strands. A hatched block in Fig. 1b indicates the region of pLmyc 10 sequenced. The nucleotide sequence was determined by the dideoxynucleotide chain termination method³⁴ essentially as described elsewhere³⁵. Certain sequences were confirmed by chemical sequencing as described by Maxam and Gilbert³⁶.



Experiments also revealed several SCLC cell line DNAs, including H378, amplified for either the 10.0-kb or 6.6-kb *Eco*RI DNA fragment (NCI lines H298, 10-fold; H378, 20-fold; H510, 15-fold; and N691, 10-fold), when compared with other SCLC cell line DNAs (Fig. 1b). In addition, a SCLC tumour sample obtained directly from a patient at autopsy showed an approximate 15-fold amplification of L-*myc* DNA when compared with the patient's normal tissue DNA (Fig. 1b). Some DNAs amplified for either the 10.0-kb and 6.6-kb *Eco*RI L-*myc* fragments show other uncharacterized hybridizing species (H378

L-*myc* gene expression was examined in a group of SCCL cell lines, including those that showed L-*myc* amplification. All cell lines amplified for the L-*myc* sequences show a 2.2-kb transcript in poly(A)⁺-selected RNA using the 1.8-kb *Sma*I-*Eco*RI fragment as a probe (Fig. 2). It is of interest that H378 poly(A)⁺

Table 1 Segregation of the human *L-myc* gene in rodent-human hybrid cells (% discordancy)

Human chromosome	Hybrid cell type		Total (n = 65)
	Human-mouse (n = 32)	Human-hamster (n = 33)	
1	0, 3	0, 3	0, 3
2	13, 9	39, 27	26, 18
3	25	30	28
4	34	24	29
5	28	24	26
6	22, 38	33, 36	28, 37
7	34	48	42
8	41	39	40
9	19	45	32
10	25	42	34
11	31	42	37
12	31, 41	30, 33	31, 37
13	38	30	34
14	47	39	43
15	25	48	37
16	53	18, 30	35, 42
17	53	21	37
18	63	39	51
19	9	24	17
20	25	36	31
21	47	42	45
22	19	45	32
X	44	55	49

Detection of the human *L-myc* gene (10.0-kb and 6.6-kb *EcoRI* fragments) correlated with the presence or absence of various human chromosomes in 65 mouse-human and Chinese hamster-human primary hybrids and subclones. Discordancy represents either the presence of a specific human chromosome in the absence of human *L-myc* or the presence of this sequence despite the absence of the chromosome in that hybrid. The *L-myc* locus can be assigned to that chromosome with the lowest discordancy. When two numbers are given, they refer to discordancy with short-arm and long-arm markers, respectively. The human-mouse hybrids consist of 15 primary clones and 17 subclones of 5 additional hybrids¹⁷⁻¹⁹ and 12 contained the human *L-myc* sequence. The hamster-human hybrids consist of 23 primary¹⁷⁻¹⁹ and 10 subclones; 12 were positive for human *L-myc*. Thirty-four of the hybrids came from crosses segregating the 10.0-kb allele, 25 came from crosses segregating the 6.6-kb allele and for the remaining 6 the RFLP pattern of the parents was not determined. The hybrid cell line DNAs also provided additional information on the subchromosomal localization of *L-myc* on human chromosome 1. One mouse-human hybrid contained the 6.6-kb *L-myc EcoRI* allele, the genes for phosphoglucomutase-1 on 1p, *N-ras* on 1p and a 6.2-kb *EcoRI* metallothionein pseudogene on 1p, but lacked the genes for peptidase C on 1q and a 2.8-kb *EcoRI* metallothionein pseudogene¹⁷. A human-hamster hybrid contained the 10.0-kb *L-myc EcoRI* allele, the 6-phosphogluconate dehydrogenase gene on 1p, phosphoglucomutase-1, enolase-1 on 1p and the metallothionein 6.2-kb sequence, but lacked the genes for peptidase C, *N-ras* and the 2.8-kb metallothionein sequence. These results strongly suggest that both the 10.0-kb and 6.6-kb *EcoRI L-myc* alleles are located on human chromosome arm 1p and are distal to the *N-ras* gene on 1p (ref. 21).

RNA shows both a 2.2-kb and a 3.8-kb RNA transcript. The origin of the novel 3.8-kb species and its relationship to the 2.2-kb RNA are unclear. One other SCLC cell line (H209) not amplified for the *L-myc* gene sequence also expresses an *L-myc* RNA transcript of 2.2 kb (see Fig. 2). Two representative SCLC lines that are amplified and express increased amounts of either *c-* or *N-myc* mRNAs (H82 and H249 respectively) contain no detectable amounts of the 2.2- or 3.8-kb *L-myc* RNA transcript (see Fig. 2). Because *c-myc*-expressing SCLC cell lines do not express *N-myc* and vice versa¹⁰, it appears that only one of these three *myc*-related genes is expressed at detectable levels in total RNA from any individual SCLC cell line thus far examined.

Previous experiments comparing *c-myc* and *N-myc* sequences have demonstrated two short regions of homology between these two genes, localized to the second exon of the *c-myc* gene⁶. To understand better the structural relationship of the *L-myc* gene to the *c-myc* and *N-myc* genes, the region of homology in *L-myc* was mapped to restriction fragments located about 100 base pairs (bp) 5' to the 1.8-kb *SmaI-EcoRI L-myc* probe (see map, Fig. 1b) and the nucleotide sequence determined. Figure 3 shows a nucleotide and predicted amino-acid comparison of the

homologous regions of the *c-*, *N-* and *L-myc* genes. Like *N-myc*, *L-myc* contains the same two regions of homology to the *c-myc* gene separated by a stretch of nucleotides that bears no resemblance to the analogous region in *c-myc*. The 5' and 3' blocks of homology show about 80% nucleotide sequence homology with the analogous regions of either *c-* or *N-myc*. An open reading frame spans the 5' and 3' blocks of homology shown in Fig. 3, including the 135-nucleotide non-homologous stretch between these two regions. The predicted amino-acid sequences for the two homology regions of *L-myc* show overall 82% and 76% homology when compared with that of the *c-myc* or *N-myc* gene respectively.

The *c-myc* gene is assigned to human chromosome 8 (refs 1, 11) while *N-myc* is assigned to chromosome 2 (ref. 12), indicating dispersion of these two genes to different autosomes. We mapped the *L-myc* gene to a human chromosome region. Southern blot analyses using the 1.8-kb *SmaI-EcoRI L-myc* probe revealed a 12-kb *EcoRI L-myc* hybridizing band in both hamster and mouse DNAs, indicating the presence of an *L-myc* homologue in these two species (Fig. 4a). Analysis of rodent-human hybrids segregating human chromosomes¹⁷⁻¹⁹ revealed either retention of the 10.0-kb or 6.6-kb fragment or neither of these human *EcoRI L-myc* fragments (Fig. 4a). A panel of 65 human-rodent hybrid clones representing 43 independent hybrids with 4 different human parents were scored for the presence or absence of human *L-myc* genes and this was correlated with the retention of each human chromosome (Table 1). Both the 10.0-kb and 6.6-kb *EcoRI L-myc* alleles segregated concordantly with human chromosome 1 and discordantly with the other human chromosomes, indicating that this autosome is the most likely location for *L-myc*.

The *L-myc* gene was also mapped using the technique of chromosome *in situ* hybridization. A total of 159 separate metaphases were analysed and the results plotted on a 400-band idiogram (Fig. 4b). Grains at 1p32 comprise 7.7% of the total grains and the probability of this distribution occurring as a random event is $P < 10^{-5}$. Thus, by both somatic cell hybridization and chromosome *in situ* hybridization techniques, the *L-myc* genes have been localized to the short arm of chromosome 1, very near or within band 1p32. This is approximately the same location to which a putative oncogene, *Blym*, was mapped²⁰ earlier but distal to the proto-oncogene *N-ras*²¹. It is interesting that the human melanoma kindred gene has also been provisionally localized to 1p (ref. 13). In addition, the *L-myc* gene and its associated *EcoRI* RFLP may prove to be a useful marker in analysing cytogenetic changes in human tumours involving the 1p32 region.

Given the well-defined and limited region of homology, it will be of great interest to see whether the three *myc* gene products have similar or related functions. SCLC cell lines that contain amplified *L-myc* DNA sequences also contain at least one *L-myc*-related RNA transcript. In addition, *L-myc* has been mapped to 1p32, a chromosomal location different from that of the *c-myc*^{1,11} or *N-myc*¹² genes. The fact that *L-myc* has been found amplified in a patient's tumour tissue, but not in the normal tissue, suggests that this gene may be important in the genesis or progression of SCLC.

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Sarcoma viruses carrying *ras* oncogenes induce differentiation-associated properties in a neuronal cell line

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The growth-promoting and/or differentiation-blocking activities of Kirsten (Ki-MSV) or Harvey murine sarcoma virus (Ha-MSV) on various types of cells *in vitro* are well documented¹⁻⁸. Here we report an unexpected effect of these viruses on a rat pheochromocytoma cell line, PC12. PC12 cells, which multiply indefinitely in growth medium, are known to respond to nerve growth factor (NGF) by cessation of cell division and expression of several properties resembling those of differentiated sympathetic neurones^{9,10}. We have found that Ki- and Ha-MSV mimic some, if not all, of the activities of NGF in PC12 cells, and there is evidence that the viral oncogenes, *v-Ki-ras* and *v-Ha-ras*, are responsible for this phenomenon. This system may be of value for studying the mechanism of action of the *v-ras* genes as well as the regulatory mechanism of growth and differentiation in neuronal cells.

In an initial attempt to 'super-transform' PC12 cells, we infected the cells with one of five mammalian retroviruses carrying different oncogenes. To our surprise, Ki- or Ha-MSV, carrying *v-Ki-ras* or *v-Ha-ras* respectively, induced marked morphological changes of the host cells within 2-3 days of infection, that is, enlargement of the cell bodies and extension of neurite-like processes (Fig. 1c, e). These changes were similar to those induced by NGF (Fig. 1b), but in this case it was more than 4 days before we observed processes of comparable length to those induced by the *ras*-containing viruses. Moloney (Mo) MSV, carrying the *v-mos* gene, seemed to induce some morphological changes in a few days after infection (Fig. 1f), but the cells subsequently became very large and granulous, and event-

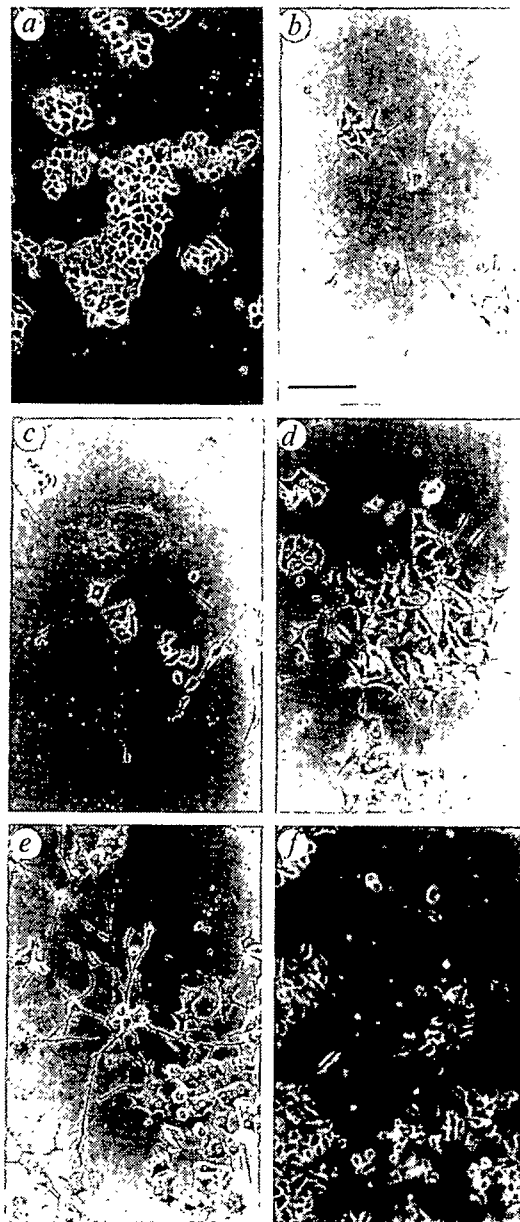


Fig. 1 Morphology of NGF-treated or MSV-infected PC12 cells. PC12 cells were usually grown at 36 °C in DMEM (Gibco) supplemented with 5% (v/v) heat-inactivated horse serum (Flow Laboratories), 5% fetal calf serum (Mitsubishi Kasei Co.), 50 U ml⁻¹ penicillin G and 50 µg ml⁻¹ streptomycin sulphate in a humidified CO₂ incubator. Virus infection was carried out in the presence of 6 µg ml⁻¹ polybrene (Aldrich). a, Control PC12 cells cultured in the above growth medium; b, PC12 cells incubated for 7 days in growth medium containing 50 ng ml⁻¹ of 2.5S NGF (Collaborative Research, Inc.); c, e, f, PC12 cells infected with Ki-, Ha- or Mo-MSV, respectively, and incubated for 4 days in growth medium; d, PC12 cells infected with the temperature-sensitive mutant of Ki-MSV and incubated for 4 days in growth medium at a non-permissive temperature, 39 °C. Scale bar, 100 µm.

ually died. Abelson murine leukaemia virus, carrying the *v-abl* gene, did not induce long processes, although the cells became spindle-shaped (not shown) and were easily detached from dishes after prolonged incubation. Simian sarcoma virus showed no apparent effect on the morphology of PC12 cells.

Under our culture conditions (see Fig. 1 legend), PC12 cells proliferated at an average doubling time of 42 h. By contrast, Ki-MSV-infected, process-extending cells stopped dividing within 3 days (Fig. 2). A similar result was obtained with Ha-MSV (not shown). The changes induced by the *v-ras*-containing MSVs do not seem to represent mere degeneration of the cells,

because the infected cells survive for many weeks, migrating actively. It was also observed that when PC12 cells were infected by Ki- or Ha-MSV and incubated for 24 h in growth medium, they could survive and extend processes in serum-free medium. The initial incubation in serum-containing medium seemed to be required for the expression of the phenotypic changes. However, cell division did not appear necessary (more rigorous experiments to explore this point are underway).

Several lines of evidence indicated that *v-ras* genes are responsible for these changes. First, the agent responsible for the changes was probably a sarcoma virus component in each virus stock, because helper MLV alone did not induce these changes, and, moreover, the number of process-extending cells induced by each of the stocks of Ki- or Ha-MSV from several different sources was roughly comparable to the number of focus-forming units measured on a rat fibroblast cell line (data not shown). It is known that only one protein is encoded by the genome of each MSV, that is, p21^{v-Ki-ras} by Ki-MSV and p21^{v-Ha-ras} by Ha-MSV¹¹. Second, transforming viruses carrying oncogenes other than *v-ras* did not induce the same type of changes (see above). Third, a temperature-sensitive (ts) mutant¹² of Ki-MSV failed to arrest the growth of PC12 cells at non-permissive temperature, although the process-inducing activity was not completely abolished (Fig. 1d). This last observation may suggest either that viral p21 proteins have more than one functional domain, or that growth-arrest requires more activity of viral p21 than does process-induction. Partial transforming activities of the ts Ki-MSV mutant at non-permissive temperature in other cell types have been reported^{4,13}.

To determine whether the changes induced by the *ras*-containing virus are involved in neuronal differentiation, we investigated whether neurone-specific properties known to be induced by NGF are also induced by the viruses in PC12 cells. First, we observed that Ki- or Ha-MSV-infected PC12 cells contained higher activities of acetylcholinesterase (as did NGF-treated cells¹⁴) than did uninfected control cells (Table 1). Second, electrophysiological examinations revealed that the mean resting potential of Ki-MSV-infected PC12 cells, as well as of NGF-treated cells, was significantly greater than that of untreated controls (Table 2). Moreover, Ki-MSV-infected, process-extending cells showed significantly higher levels of action potentials than did control cells. Judged from the two components of action potentials (that is, low and high threshold potentials), both Na⁺ and Ca²⁺ channels seemed to develop

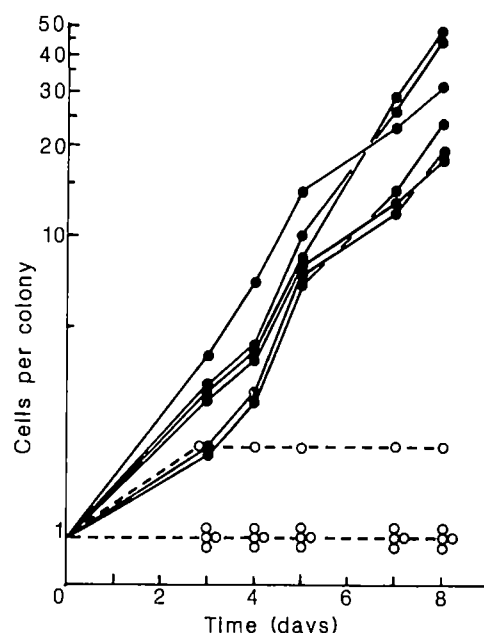


Fig. 2 Growth curve of Ki-MSV-infected or uninfected PC12 cells. PC12 cells were infected in suspension with Ki-MSV (MOI = ~1) and plated into a 96-well dish at ~1 cell per well with growth medium. As a control, uninfected PC12 cells were plated in the same way. After 16 h, wells containing a single cell were marked and the numbers of cells in each well were scored under a phase-contrast microscope at 24–48-h intervals. ●, Uninfected cells; ○, Ki-MSV-infected, process-extending cells.

after Ki-MSV infection, although to a lesser extent than those induced by NGF¹⁵ (Table 2).

The above results indicate that at least some of the properties associated with the induction of neuronal differentiation by NGF are also induced by Ki- or Ha-MSV in PC12 cells. This is in marked contrast to the previously known biological activities of these viruses *in vitro*, which include malignant transformation of certain cells^{1–5}, blockade of Ca²⁺-dependent differentiation of primary⁶ as well as established mouse keratinocytes⁷, and growth stimulation of differentiation-competent mouse erythrogenic cells⁸. We have observed that rat

Table 1 Acetylcholinesterase activities in PC12 cells treated with NGF or infected by Ki- or Ha-MSV

	Acetylcholinesterase activity (nmol per min per mg protein)		
	Control	NGF	Ki-MSV
Expt 1			
39.6 ± 2.2	56.1 ± 3.3	55.4 ± 2.1	ND
Expt 2			
32.8 ± 0.7	42.7 ± 1.3	68.2 ± 1.1	46.5 ± 0.72
Expt 3			
a, In growth medium:			
24.5 ± 1.0	35.5 ± 0.8	28.1 ± 0.7	ND
b, In serum-free medium:			
ND*	44.2 ± 1.5	47.0 ± 1.6	ND

PC12 cells (3×10^5 cells per 60-mm dish) were infected with Ki-MSV (multiplicity of infection (MOI) ~1) and incubated for 5 days in growth medium (expts 1, 2, 3a) or 1 day in growth medium, then 4 days in serum-free Dulbecco's modified Eagle's medium (DMEM) (expt 3b). As controls, the same number of cells were cultured in growth medium with or without 50 ng ml⁻¹ of 2.5S NGF. The cells were collected and homogenized as described elsewhere¹⁴. A colorimetric enzyme assay was performed with homogenates from triplicate cultures for each group by the method of Ellman *et al.*²⁶. Data are given as mean ± s.e. ND, not determined.

* Control PC12 cells did not survive in serum-free medium.

Table 2 Electrophysiological properties of PC12 cells treated with NGF or infected with Ki-MSV

	Control (n = 8)	NGF (n = 11)	Ki-MSV (n = 15)
Resting membrane potential (mV)			
-22.9 ± 1.0	-28.4 ± 0.33	-27.3 ± 1.6	
Time derivative of action potential (V s ⁻¹)			
Low threshold (-20 mV):			
0	2.70 ± 0.84	1.21 ± 0.37	
High threshold (0 mV):			
0.64 ± 0.43	3.89 ± 0.40	2.20 ± 0.45	

PC12 cells (1×10^5) were infected in suspension with Ki-MSV and plated in 35-mm poly-D-lysine-coated plastic dishes with growth medium. The same number of uninfected cells were plated in medium with or without 50 ng ml⁻¹ NGF. After 8 days of incubation at 36 °C, the culture fluid was replaced with a recording buffer (130 mM NaCl, 5.4 mM KCl, 1.8 mM CaCl₂, 0.8 mM MgSO₄, 5.5 mM glucose and 10 mM HEPES, pH 7.3), and electrophysiological examination using a microelectrode was performed as described elsewhere²⁷. Cells of typical sizes and morphologies were impaled and hyperpolarized to -100 mV for 1 s, then action potentials were triggered by injecting 50-ms depolarizing current pulses. Two components with threshold potentials of about -20 mV (representing activity of voltage-dependent Na⁺ channels) and about 0 mV (activity of voltage-dependent Ca²⁺ channel) were recognized in the time derivative of action potential, and are listed separately here. In control cells, a low-threshold action potential was not evolved. Results are given as mean ± s.e.

C6 glioma, a cell line whose origin was more closely related to that of PC12, was also growth stimulated by these MSVs (unpublished observation). Therefore, the growth-arresting and differentiation-inducing effects of Ki- and Ha-MSV may be highly specific to either this particular cell line or, possibly, to neuronal cells in general.

In experiments to investigate the mechanism of action of this phenomenon, we observed that anti-NGF serum of reasonably high titre failed to inhibit the process-extension and growth-arrest induced by Ki-MSV. Also, we have not detected any biologically active factors in conditioned medium collected from Ki- or Ha-MSV-infected PC12 cultures. We therefore believe, although this has not been proved, that some intracellular reactions induced by viral p21, rather than endogenously secreted differentiation factors, may trigger the phenotypic changes described above.

Very recently, two groups of investigators have discovered phenomena related to our observation. First, D. Bar-Sagi and J. Feramisco found that the transforming Ha-ras protein—but not its normal counterpart—introduced into PC12 cells via microinjection induces process-extension and arrest of DNA synthesis (personal communication), providing further evidence that the activated p21 itself is indeed responsible for this phenomenon. Second, Alemà *et al.* found that Rous sarcoma virus, which carries the *v-src* gene, induces changes almost identical to those described here in PC12 cells¹⁶. However, the kinetics of process-induction by *v-ras* and by *v-src* seem to differ from each other; *v-ras* induces processes with a shorter lag time and at a higher rate than either NGF or *v-src*. Although the significance of this difference and the relationship of these two analogous phenomena are unclear, one possibility is that *v-ras* and *v-src* share a common pathway of action in this system. Previously, we have found that two clones of flat revertants isolated from a Ki-MSV-transformed fibroblast cell line were resistant to 'retransformation' by *v-src*-containing viruses as well as by *v-ras*-containing viruses¹⁷, suggesting some functional relationship between the two oncogenes in transformation of fibroblasts also.

The mechanism of action of NGF remains controversial^{18,19}. Hypotheses have been proposed to take account of second messengers such as cyclic AMP and Ca²⁺ (ref. 20), as well as of other signal-transducing mechanisms^{21–23} and direct translocation of the ligand-receptor complex into the cell nucleus^{18,19}. Examination of such early physiological changes in PC12 cells infected by either *v-ras*- or *v-src*-containing viruses should provide useful information on the mechanisms of action of the viral oncogenes as well as of NGF itself. To examine the role of cyclic AMP in this system is of special interest, in view of the recent discovery that the *ras*-related genes in yeast seem to serve as GTP-dependent regulatory components of adenylate cyclase^{24,25}.

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Deletion of Huntington's disease-linked G8 (D4S10) locus in Wolf-Hirschhorn syndrome

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Huntington's disease (HD) is an autosomal dominant neurodegenerative disorder characterized by progressive involuntary movements and dementia^{1,2}. The symptoms of the disease, although devastating in severity, do not usually appear until the third to fourth decade of life. The gene defect is highly penetrant, and results in the loss of neurones in the basal ganglia, globus pallidus, and more diffusely in the cortex. A DNA marker, G8 (or D4S10), is tightly linked to Huntington's disease and this gene has been localized to chromosome 4 (ref. 3). The discovery of this linkage marker raises the possibility of developing a presymptomatic test for the disorder, and of eventually isolating the disease gene based on its map position⁴. We have now regionally localized the DNA marker G8 to the terminal band of the short arm of the chromosome, a region representing approximately 0.5% of the total human genome. The assignment was made by examining DNA from patients with Wolf-Hirschhorn syndrome, a birth defect resulting from partial heterozygous deletion of the short arm of chromosome 4.

Chromosome 4 contains approximately 6.55% of the total human genomic DNA. The search for additional DNA fragments near the HD gene and for the HD gene itself would be facilitated if the G8 marker were more precisely localized to only a small region of the chromosome. To achieve this goal, we used cells from patients afflicted with Wolf-Hirschhorn syndrome (WHS), a congenital anomaly involving heterozygous deletion of part of the short arm of chromosome 4 (4p-syndrome)^{5–7}. Victims of WHS are profoundly mentally retarded and have a characteristic craniofacial appearance including microcephaly, arched eyebrows, ocular hypertelorism, broad malformed nose, lobeless ears and micrognathia. The loss of chromosome 4 material can be the result of a *de novo* deletion, or due to inheritance of an unbalanced translocation, but most commonly involves the terminal 4p16 band⁷.

Initially, we obtained blood samples from six independent victims of WHS to establish permanent lymphoblastoid cell lines, and to prepare genomic DNA. A fibroblast line, GM0343, from a seventh WHS patient was obtained from the NIGMS

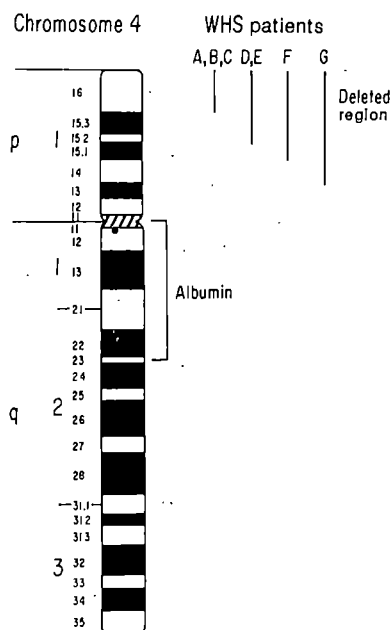


Fig. 1 Extent of chromosome 4 deletion in Wolf-Hirschhorn syndrome (WHS) patients. The region of chromosome 4 deleted in each patient, as determined by standard trypsin-Giemsa banding techniques, is shown. Note that the application of more sophisticated prophase banding methods could potentially detect differences between the deletions in patients A, B and C. The region containing the albumin locus is taken from ref. 17.

Human Genetic Mutant Cell Repository, Camden, New Jersey⁸. These patients presented a range of symptoms characteristic of WHS, and each had suffered an apparent terminal deletion of 4p. The extent of deletion in each WHS victim based on standard trypsin-Giesma banding is depicted in Fig. 1. In the case of patient D, the deleted 4p region had been replaced by an unidentified segment of another chromosome⁹. Although the size of the missing region was not identical in each case, all

patients were monosomic for at least band 4p16 which represents about 1/15 of the chromosome. For patients A, B and D, both parents were available for study. Blood samples could not be obtained from parents of the other cases. For patient A, an unaffected brother was also sampled.

The genetic linkage of G8 to HD was originally achieved using two restriction fragment length polymorphisms (RFLPs) detected by this probe in human genomic DNA digested with *HindIII*³. The informativeness of the G8 region as a genetic marker has recently been expanded by the cloning of adjacent genomic sequences and the discovery of additional RFLPs with the enzymes *BglI* (two sites), *EcoRI*, *PstI*, and *NciI* (ref. 10 and J.F.G., R.E.T. and R. A. Heft, manuscript in preparation). We reasoned that if the G8 locus mapped outside the deleted region, this could be demonstrated by detecting heterozygosity at any of these seven restriction enzyme sites. On the other hand, if the G8 region were deleted in a given individual, heterozygosity should never be observed. In addition, the apparent genotype of the WHS victim in such a case might be incompatible with the inheritance of one allele from each parent. We therefore performed Southern blot experiments with genomic DNA from each of the seven WHS patients, the three available sets of parents and the normal sibling of patient A. DNA was digested with each of the enzymes listed above, fractionated by agarose gel electrophoresis, transferred to a nylon filter and hybridized with appropriate probes known to reveal each RFLP. As a control, the DNAs were also typed for a *PstI* polymorphism at the albumin locus which maps below the centromere on chromosome 4¹¹. The results of this analysis are shown in Table 1.

Four of the seven WHS patients were heterozygous at the albumin locus indicating, as expected, that they were not deleted for this gene on the long arm of chromosome 4 and this polymorphism displays a level of heterozygosity of 49% in the general population. None of the seven patients was heterozygous at any of the RFLPs in the G8 region, although each of the normal parents was heterozygous for at least one of the seven sites. Table 1 also shows the level of heterozygosity displayed by each of these RFLPs in a random sample of 50 North Americans. Overall, 92% of unrelated individuals are heterozygous for at least one restriction enzyme site using this set of

Table 1 Phenotypes observed for RFLPs at the albumin and G8 loci

Individual	Cell line	Albumin locus <i>PstI</i>	G8 locus							Heterozygous at G8 locus
			<i>HindIII</i> (no. 1)	<i>HindIII</i> (no. 2)	<i>BglI</i> (no. 1)	<i>BglI</i> (no. 2)	<i>EcoRI</i> (no. 1)	<i>NciI</i>	<i>PstI</i>	
Patient A	GUS 2305	12	1	1	2	2	1	1	1	No
Father A	GUS 2302	12	1	1	1	2	2		12	Yes
Mother A	GUS 2303	12	1	1	12	2	1		1	Yes
Brother A	GUS 2304	12	1	1	1	2	12		12	Yes
Patient B	GUS 1548	12	1	1	1	2	1	1	1	No
Father B	GUS 1549	2	1	1	12	2	12		1	Yes
Mother B	GUS 1547	12	12	1	12	2	1		1	Yes
Patient C	GUS 1654	12	2	1	1	1	2	1	2	No
Patient D	GUS 2085	1	2	1	2	2	2	2	1	No
Father D	GUS 2225	12	12	12	2	2	2		1	Yes
Mother D	GUS 2224	1	12	12	2	2	12		1	Yes
Patient E	GUS 2098	1	2	1	1	2	2	2	1	No
Patient F	GUS 1553	12	1	1	2	2	2	1	1	No
Patient G	GM 0343	1	2	1	1	1	1	2	2	No
Heterozygosity		49%	46%	36%	46%	22%	58%	38%	16%	92%

Phenotypes for each RFLP were scored from the pattern of fragments observed on an autoradiogram after hybridization of Southern blots (containing genomic DNA digested with the indicated enzyme) to probes known to detect polymorphisms at either the albumin or the G8 locus (refs 3, 10 and J.F.G., R.E.T. and R. A. Heft, in preparation). Alleles are numbered 1 and 2 in order of decreasing size except in the case of *HindIII* site 2, where the order is reversed³. Heterozygotes at a particular site therefore have the phenotype 12. Heterozygosity in the general population, shown in the last line of the table, was calculated for albumin from ref. 16. For the G8 RFLPs, both individually and in combination, the table shows the level of heterozygosity observed in a random sample of 50 North Americans. All GUS cell lines are lymphoblastoid lines initiated in our laboratory for preparation of DNA³. GM 0343 is a fibroblast line purchased from the Human Genetic Mutant Cell Repository⁸.

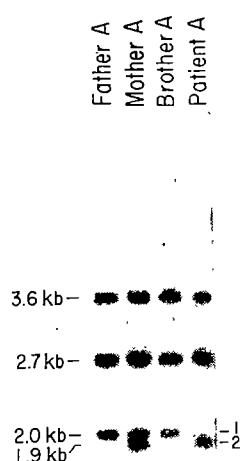


Fig. 2 Deletion of the G8 locus in patient A. DNA from patient A, his brother, and both parents was digested with *Bgl*I and fractionated by agarose gel electrophoresis (0.8% agarose, 3 V cm⁻¹, 18 h). The DNA was transferred to Zetapor nylon membrane (AMF/Cuno) and hybridized to ³²P-labelled probe R7¹⁰. R7 is a phage clone containing a 16-kb fragment of chromosome 4 that partially overlaps with the G8 phage insert. Together, the two clones comprise approximately 29 kb of adjacent sequence from chromosome 4 (J.F.G., R.E.T. and R. A. Heft, in preparation). Fragments representing alleles 1 (2.0 kb) and 2 (1.9 kb) are indicated on the figure. All other fragments detected by the probe are invariant.

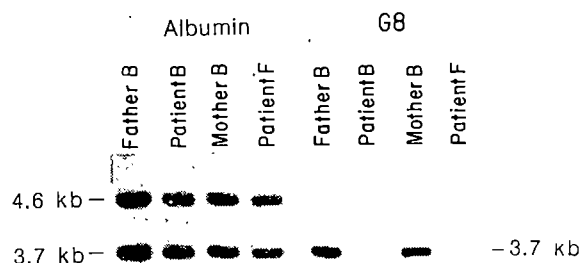


Fig. 3 Detection of hemizygosity for G8 locus by dosage. DNA (5 µg) from patient B, patient F, and the normal parents of patient B were digested with the enzyme *Hind*III and fractionated by electrophoresis on a 1% agarose gel. The DNA was transferred to Zetapor membrane and hybridized to ³²P-labelled pK082, a plasmid subclone of the G8 insert^{10,12}. This probe hybridizes to a single fragment of 3.7 kb in the size range examined. After autoradiography to detect the pK082 hybridization, the probe was removed from the filter by washing for 2 h at 65 °C in 0.01 × SSC (1 × SSC = 0.15 M NaCl, 0.015 M Na-citrate). The filter was then hybridized to ³²P-labelled pcHSA33-1, a human albumin cDNA clone¹³. This probe detects two fragments of 4.6 kb and 3.7 kb. Both probes detect additional fragments >10 kb in a *Hind*III digest, but these were not compared in these experiments due to difficulties in obtaining uniform transfer of large restriction fragments. The fact that both probes detect small fragments of similar size (3.7 kb) allows direct comparison of relative dosage without the ambiguities introduced by comparison of fragments that are very different in size.

seven RFLPs. The probability of picking seven individuals who are homozygous for all sites is therefore $(0.08)^7 = 2 \times 10^{-8}$. Thus, based on the lack of heterozygosity alone, it is likely that many or all of these WHS victims are in fact hemizygous at the G8 locus.

In patient A, the G8 locus has definitely been deleted from one chromosome (Fig. 2) as the child's DNA shows only the 1.9-kilobase (kb) *Bgl*I band representing the 2 allele. The genotypes of the father, mother and brother are 1 1, 1 2 and 1 1, respectively. Therefore, the WHS child is hemizygous at the G8 locus, having received a 2 allele from the mother with no contribution from the father. Hemizygosity at the G8 locus in patient A can also be demonstrated using the *Eco*RI RFLP (Table 1). For patients B and D where parental DNAs were also available, it was not possible to unequivocally demonstrate deletion of G8 by genotype alone. For these two, and for the patients with no available parents, we have compared the intensity of hybridization to sequences from the G8 region with that to a probe for albumin, which maps to the long arm of chromosome 4 (4q). Examples of these dosage experiments are shown in Fig. 3. A filter containing *Hind*III-digested DNA from patient B and his parents and patient F, was hybridized sequentially to pK082, a subclone of G8, and to pcHSA33-1, an albumin cDNA^{12,13}. *Hind*III was chosen for this experiment as both probes detect a fragment in the 3.7-kb range, thereby eliminating the possibility of artefact due to unequal transfer to the filter of DNA fragments of widely divergent sizes. In both patients, the intensity of hybridization of the albumin probe was approximately equal to that seen in the two normal parents. With G8, however, the intensity of hybridization was markedly reduced in the WHS children, indicating hemizygosity at the G8 locus. Similar experiments (data not shown) using *Hind*III and other restriction enzymes suggest hemizygosity at the G8 locus in each of the seven WHS victims. Ultimate confirmation of these results could be obtained by *in situ* hybridization experiments, or by segregating the deleted chromosomes in somatic cell hybrids. We are currently pursuing the latter strategy since it will generate cell lines with which new DNA probes can be quickly mapped to the vicinity of the HD gene.

The assignment of G8 to 4p16 does not dictate that the HD locus is also contained in this band. As the two are tightly linked,

however, at a distance considerably less than 10 centimorgans (10% recombination), it is likely that the HD gene maps to either 4p16 or to the distal portion of 4p15^{3,4,14}. More precise assignment of the HD gene will probably result from the isolation of another polymorphic DNA marker on the opposite side of the disease gene from G8. Such a flanking marker would make it possible to bracket the HD gene and physical mapping of the new marker relative to G8 would then give a precise position for the HD locus⁴. The isolation of a flanking marker is also essential for the development of an accurate presymptomatic and prenatal linkage test for HD¹⁵. With only the G8 marker, it is impossible to eliminate potential errors in diagnosis that would be caused by the recombination events that can occur between the HD locus and G8. Choosing DNA fragments at random from chromosome 4 has thus far been unsuccessful in providing the needed flanking marker. The mapping of G8 to the region deleted in WHS victims has presented a new, more efficient strategy. Segregation of the deleted chromosomes from WHS patients in somatic cell hybrids will provide the material necessary to rapidly screen new DNA clones to determine whether they map to 4p16, which represents only about 1/15 of the chromosome. Selective hybridization schemes based on these cell lines might also yield large amounts of DNA from this band, and provide a tremendous boost for efforts to clone and characterize the HD gene itself. Given the proximity of the G8 and HD loci, at least some of the WHS patients, especially patient F, must carry a deletion and therefore be hemizygous for the normal counterpart of the defective Huntington's disease gene. If the HD mutation causes the inactivation of a gene, these patients might be the functional equivalents of an HD heterozygote with a clinical picture clouded by the effects of other hemizygous loci. Since WHS victims rarely live to an advanced age and HD is normally a late-onset disorder, it is not possible to make such a conclusion at this time. Long-term follow-up and careful neurological examination of older WHS patients who have been tested for deletion of the HD gene by DNA analysis might certainly be warranted, as would attempts to obtain neuropathological information on this syndrome. If the HD mutation does not cause inactivation of the gene, hemizygosity at this locus might still contribute in some way to the symptoms of WHS. A resolution of this issue will probably await

the further characterization of both disorders at the molecular level.

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Fission yeast *Schizosaccharomyces pombe* correctly excises a mammalian RNA transcript intervening sequence

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Study of heterologous gene expression in the budding yeast *Saccharomyces cerevisiae* has shown that this organism is incapable of correctly removing intervening sequences from transcripts of higher eukaryotic genes¹⁻³. This is probably due to the stringent requirement for the presence of a TACTAAC box close to the 3' end of the intervening sequence if splicing in *S. cerevisiae* is to occur⁴⁻⁶. Comparison of the introns found in the fission yeast *Schizosaccharomyces pombe*⁷⁻¹⁰ has identified conserved sequences similar to those found in higher eukaryotes¹¹. Therefore, we have investigated whether *Schiz. pombe* is capable of accurately excising intervening sequences from the transcripts of higher eukaryotic genes. We show here that both the 5' and 3' splice sites of the simian virus 40 (SV40) small-T antigen transcript are accurately utilized when cloned viral DNA is expressed in *Schiz. pombe* cells. These data suggest that *Schiz. pombe* may be a better model system than *S. cerevisiae* for the genetic study of RNA splicing and for expressing higher eukaryotic genes.

The fission yeast *Schiz. pombe* is as convenient for molecular genetic analysis and gene manipulation¹² as the better-known budding yeast *S. cerevisiae*, but has been rather neglected in studies of eukaryotic gene expression. Recently it has become clear that *Schiz. pombe* genes generally have more introns than those of *S. cerevisiae* and that the conserved sequences in the vicinity of intron-exon boundaries are more similar to those found in higher eukaryotes. The consensus sequences found so far for *Schiz. pombe* introns are shown in Table 1. These are less stringent than those found in *S. cerevisiae*, particularly with respect to the TACTAAC box near to 3' splice sites, but are

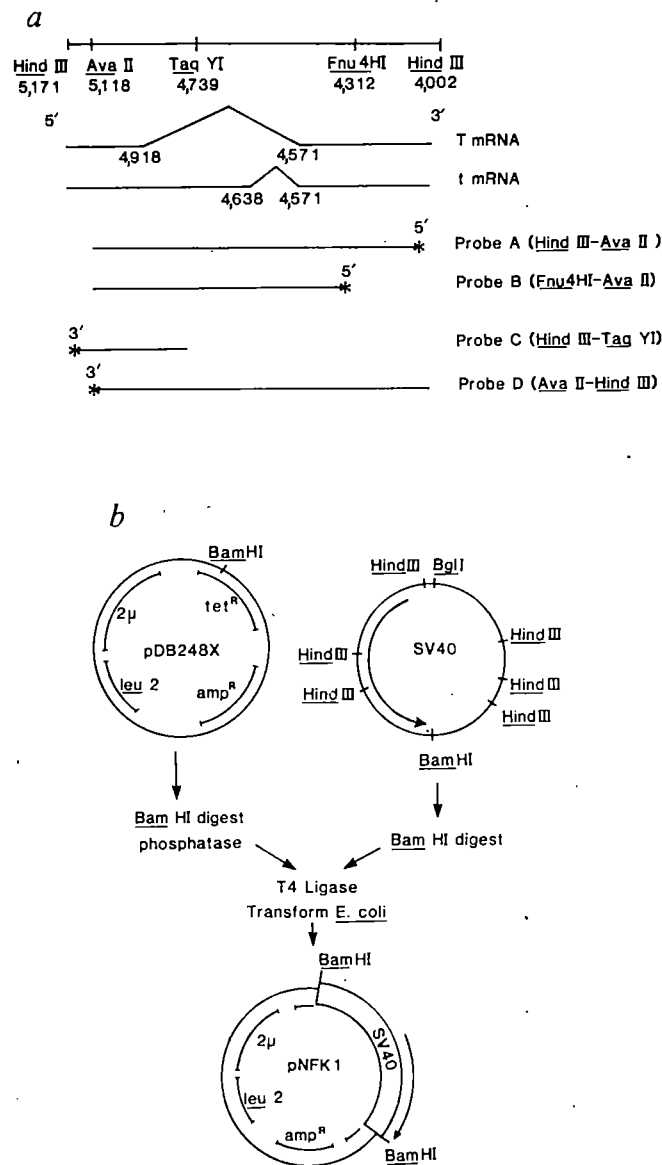


Fig. 1 a, Construction of plasmid pNFK1. The structure of the SV40 early region mRNAs within the 1,169-bp *Hind*III B fragment of SV40 is shown. The nature of the probes used in this study is also indicated. In each case, the site of labelling is given first. The numbering system used is that suggested in ref. 13. Asterisks indicate sites of labelling. b, Structure of SV40 early region mRNAs. pDB248X DNA was digested to completion with *Bam*HI, treated with calf intestinal alkaline phosphatase to prevent self-ligation, and ligated to *Bam*HI-digested SV40 DNA. The ligation mix was used to transform competent *Escherichia coli* DH-1 to ampicillin resistance. Tetracycline-sensitive clones were identified by replica plating and the DNA extracted from the transformants and analysed by digestion with *Eco*RI to determine the orientation of SV40 DNA in the plasmid. Standard techniques²³ were used throughout. All enzymes were purchased from Boehringer Mannheim and used according to the manufacturer's recommendation. The arrow on the SV40 genome indicates the direction of transcription of the early region. The structure of pNFK1 is shown. Similar results were obtained with pNFK2 in which the orientation of the SV40 DNA is reversed.

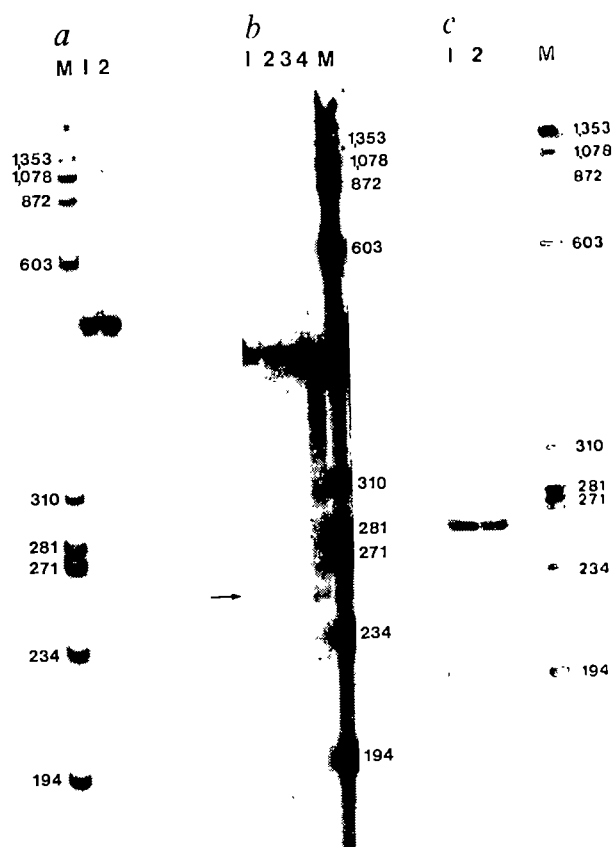
very similar to those observed in higher eukaryotes. Given these similarities, it is possible that transcripts from higher eukaryotic genes may be correctly spliced in *Schiz. pombe*.

To test this possibility, we have introduced SV40 DNA into *Schiz. pombe* and investigated the splicing of the early region, which encodes the small-T and large-T antigens. The messenger RNAs coding for these two proteins are produced by differential splicing of a precursor mRNA. They share the same 3' splice site but use different 5' splice sites (ref. 13 and Fig. 1a). SV40 DNA digested with *Bam*HI was ligated into the *Schiz. pombe* shuttle vector pDB248X, and the resultant molecule, pNFK1 (Fig. 1b), was transformed into a *Schiz. pombe* *leu1-32 h⁻* strain

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Fig. 2 S_1 mapping of 5' and 3' splice sites of SV40 early region transcripts in *Schiz. pombe*. **a**, Mapping of 5' splice sites using probe D. Lanes 1, 2, 10 μ g poly(A)⁺ RNA from cells transformed with pNFK1, S_1 digestion at 37 °C and 22 °C, respectively. Exposure was for 16 h at -70 °C with preflashed film and intensifying screen. M, DNA size marker consisting of Φ X174, digested with *Hae*III and 5'-end labelled with T4 polynucleotide kinase. **b**, Mapping of 5' splice sites using probe C. Lanes 1-3, 20 μ g, 15 μ g and 10 μ g, respectively, of total RNA extracted from SV40-infected CV-1 cells; lane 4, 10 μ g of poly(A)⁺ RNA from cells transformed with pNFK1. S_1 digestion was at 37 °C. Exposure was at -70 °C for 14 days with an intensifying screen. The arrow indicates the position of the 257-nt band. **c**, Mapping of 3' splice site using probe B. Lanes 1, 2, 10 μ g of poly(A)⁺ RNA from cells transformed with pNFK1. S_1 digestion was at 37 °C. Exposure was overnight at -70 °C with an intensifying screen.

Methods. RNA was extracted from cells in mid-exponential growth as follows. Cells were collected by centrifugation, washed once in ice-cold buffer A (50 mM HEPES-NaOH, pH 7.9, 5 mM EDTA, 150 mM NaCl) and resuspended in buffer A at $\sim 2 \times 10^9$ cells ml⁻¹. An equal volume of acid-washed glass beads (0.5 mm diameter; Sigma) was added and the cells broken by vigorous vortexing for 2 min. The liquid was removed to a fresh tube and the beads washed three times with buffer A. The combined lysate and washings were centrifuged briefly to remove cell debris and the supernatant fraction, adjusted to 1% (w/v) SDS and 100 μ g ml⁻¹ proteinase K (Sigma). After incubation at 37 °C for 30 min, the sample was extracted twice with phenol and chloroform, and precipitated with ethanol. Polyadenylated RNA was selected as described elsewhere²³ by two rounds of affinity chromatography on oligo(dT)-cellulose. DNA was extracted from SV40-infected CV-1 cells 72 h after infection at a multiplicity of 10 plaque-forming units per cell²³. S_1 mapping was performed essentially as described elsewhere²⁴. Annealing was overnight at 48 °C in 65% formamide, using 50 ng of probe, typically 20,000-50,000 c.p.m. Digestion was for 30 min at the indicated temperature using 400 U ml⁻¹ S_1 nuclease (BRL). 5'-End-labelled probes were prepared using T4 polynucleotide kinase following treatment with alkaline phosphatase. 3'-End-labelled probes were prepared using avian myeloblastosis virus reverse transcriptase. Standard techniques for labelling and subsequent gel purification were used²³. Samples were analysed on 0.3-mm-thick polyacrylamide gels²⁵ which were fixed and dried before exposure.



by selecting for *leu*⁺ prototrophic transformants. Polyadenylated RNA was prepared from a culture of one of these transformants in mid-exponential growth. A Northern blot of this RNA was probed with the 1.1-kilobase (kb) *Hind*III B fragment of SV40 which covers the early gene region (Fig. 1a). Transcripts hybridizing to this probe were detected, demonstrating that the transformants contained SV40 DNA and that the early region was being expressed. Transcript mapping suggested that they were being initiated at the SV40 early promoter. Some premature termination of transcription was also observed. No transcripts hybridizing to this probe were observed using RNA from cells transformed with pDB248X (data not shown). To determine if the SV40-specific transcripts were being correctly spliced, S_1 nuclease mapping experiments were undertaken.

In order to map the 5' splice sites of the transcripts, a 1,120-base pair (bp) *Ava*II/*Hind*III fragment was 3'-end labelled at the *Ava*II site using reverse transcriptase (Fig. 1a, probe D). Two fragments should be protected from S_1 digestion if both 5' splice sites are used, one of 484 nucleotides (nt) corresponding to the small-T transcript, and one of 204 nt corresponding to the large-T transcript. The predominant band observed is at 484

nt (Fig. 2a), demonstrating accurate use of the small-T antigen mRNA 5' splice site. However, no band was seen at 204 nt, suggesting that the large-T donor site is used very inefficiently, if at all. Some very weak bands of higher relative molecular mass (M_r) were also seen on prolonged exposure, consistent with the presence of a small amount of unspliced RNA. To confirm this result a second, shorter probe was used. A 432-bp *Hind*III/*Taq*I fragment, 3'-end labelled at the *Hind*III site, was prepared (Fig. 1a, probe C). Full-length probe should be protected if the small-T antigen 5' splice site is used. To test whether the large-T antigen 5' splice site is used at all, the autoradiograph was overexposed to see if the predicted 257-nt band could be observed. Many weak bands became visible, one of which was 257 nt long; this band co-migrated with a protected fragment corresponding to the large-T antigen mRNA 5' splice site obtained using RNA extracted from SV40-infected CV-1 cells (Fig. 2b). This suggests that the large-T antigen mRNA 5' splice site may be used, although at an extremely low level.

To map the 3' splice site, which is common to transcripts of both large-T antigen and small-T antigen, a 810-bp *Ava*II/*Fnu*4HI fragment, 5'-end labelled at the *Hind*III site, was used as a probe (Fig. 1a, probe B). A 260-nt fragment was protected from S_1 -nuclease digestion (Fig. 2c), indicating that the 3' acceptor site is accurately used by *Schiz. pombe*. In addition to the 260-nt band, another, less intense band at 810 nt was observed corresponding to protection of full-length probe. This may be due to DNA-DNA reannealing or to a fraction of the transcripts not being processed. Correct use of the 3' splice site was confirmed using a *Hind*III/*Ava*II fragment 5'-end labelled at the *Hind*III site (Fig. 1a, probe A), when the expected protected fragment of 570 nt was observed (data not shown).

To determine whether the large-T or small-T antigen proteins were being synthesized, a Western blot of protein extracted from *Schiz. pombe* cells transformed with either pDB248X or pNFK1 was probed with pAb419 (ref. 14), a monoclonal antibody which recognizes an epitope found in the N-terminal region common to both proteins. Synthesis of the 20,000- M_r small-T antigen was clearly detectable in cells transformed with pNFK1 (Fig.

Table 1 Conserved sequences at intron-exon boundaries

	5' Splice site	Internal conserved sequence	3' Splice site
Higher eukaryotes	G GTAAGT G	CTAAT G C	TAG
<i>Schiz. pombe</i>	G GTANGT	CTAAT G C	TAG A
SV40 large-T	G GTATTT	CTAAT	TAG
SV40 small-T	G GTAAAT	CTAAT	TAG

The consensus for *Schiz. pombe* was derived from refs 7-10, 22 and that for higher eukaryotes from ref. 11. The SV40 sequence was taken from ref. 13.

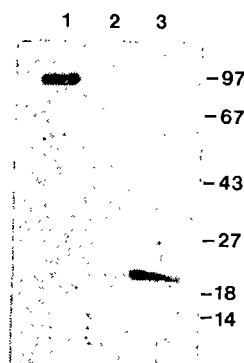


Fig. 3 Western analysis of proteins synthesized in *Schiz. pombe* transformants. Lane 1, 10 ng of purified SV40 large-T antigen; lane 2, protein extracted from cells transformed with pDB248X; lane 3, protein extracted from cells transformed with pNFK1. The numbers indicate the positions of marker proteins ($M_r \times 10^{-3}$).

Methods. *Schiz. pombe* cells transformed with the indicated plasmids were grown to a cell density of 5×10^6 cells ml^{-1} in minimal medium. The cells were collected by centrifugation, washed once in phosphate-buffered saline and broken by vortexing with glass beads. The broken cells were boiled in sample buffer²⁶ for 2 min. Cell debris was then removed by centrifugation, and the protein extracts were loaded onto a 5–15% (w/v) polyacrylamide gel²⁶. After electrophoresis the protein was transferred to nitrocellulose²⁷. The blot was hybridized with pAB419 (ref. 14) as described elsewhere²⁸. After washing, bound antibody was detected using ¹²⁵I-protein A (Amersham). The blot was autoradiographed using preflashed film and an intensifying screen at -70°C .

3). However, even on prolonged exposure, no large-T antigen was detected, a result consistent with the observed splicing pattern of the SV40 early region transcripts made in *Schiz. pombe*.

These results demonstrate that *Schiz. pombe* can correctly recognize the 5' and 3' splice sites of the SV40 small-T antigen pre-mRNA. The correct 3' splice site is not recognized in the budding yeast *S. cerevisiae* and the 5' splice sites utilized only very inefficiently¹⁵. In *Schiz. pombe* the 5' splice site used to produce the large-T antigen mRNA was used very inefficiently, if at all—an unexpected result, since in lytically infected monkey cells the large-T antigen 5' splice site is used two to three times more frequently than the small-T antigen 5' splice site¹⁶. This could be due to the difference in sequence between the SV40 large-T antigen 5' splice site and the *Schiz. pombe* consensus sequence (Table 1). None of the *Schiz. pombe* introns sequenced to date has a thymine at nucleotide 5 at the 5' end. Alternatively, it is conceivable that although *Schiz. pombe* can recognize higher eukaryotic splicing signals, it cannot cope with multiple 5' splice sites for a single 3' splice site. Thus, having identified the 3' splice site, perhaps through an interaction with the internal conserved sequence, the *Schiz. pombe* cell may scan through the transcript until it identifies the first 5' splice site and completes intron excision. A second 5' splice site beyond this would then be used inefficiently, if at all. In the SV40 system, this possibility is readily testable using the $\Delta 54$ –59 mutants of SV40¹⁷, in some of which the small-T antigen 5' splice site is deleted.

Another observation which may be relevant to this phenomenon is the report that *Xenopus* oocytes use only the small-T 5' splice site when SV40 genomes are co-injected with antibodies against ribonucleoprotein particles¹⁸. This observation suggests that the two 5' splice sites may have different properties. It is thus possible that use of the large-T 5' splice site may have a different requirement for ribonucleoprotein particles from that of the small-T 5' splice site which cannot be provided by the heterologous *Schiz. pombe* cells.

This is the first report of a higher eukaryotic intron being correctly spliced in yeast. Although it may initially seem odd that fission yeast should behave so differently from budding yeast, it should be remembered that these two organisms, although both ascomycetes, are not closely related. Recent sequence comparisons suggest an evolutionary divergence which could be as much as 1,200 Myr¹⁹. There are also differences in their promoters²⁰ and in aspects of the mitotic cycle such as the

presence of a G_2 period and chromosome condensation²¹. In all cases, the behaviour of *Schiz. pombe* is closer to that of higher eukaryotes than is *S. cerevisiae*. For these reasons we suggest that *Schiz. pombe* should be considered more seriously as an organism for expressing higher eukaryotic genes and for genetic analysis of eukaryotic splicing.

We thank ICRF for financial assistance, EMBO for a short-term fellowship awarded to N.F.K., and our colleagues in the Cell Cycle Control Laboratory for helpful discussions.

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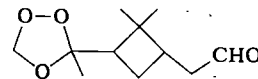
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Corrigendum

Hydroxymethyl hydroperoxide and bis(hydroxymethyl) peroxide from gas-phase ozonolysis of naturally occurring alkenes

S. Gäb, E. Hellpointer, W. V. Turner & F. Korté *Nature* **316**, 535–536 (1985)

The structure of an ozonide used to clarify the reaction pathway was drawn without a necessary methyl group. The product in equation (10) should have the following structure:



Erratum

Similarity of vaccinia 28K, v-erb-B and EGF receptors

H. R. Chen & W. C. Barker *Nature* **316**, 219–220 (1985)

IN this item of Scientific Correspondence on similarities of receptor proteins, ref. 5 should be shown on line 6 of the second paragraph. In addition the authors' address was omitted. It should read: Protein Identification Resource, National Biomedical Research Foundation, Georgetown University Medical Center, 3900 Reservoir Road, N.W. Washington, DC 20007, USA.

Sedimentary microrhythms

WITH regard to the interesting article by House¹ in which sedimentary microrhythms from marine Jurassic strata of southern England were investigated, the following may be of additional interest.

House assigned 60 ammonite zones to the Jurassic period, of which two were referred to the non-marine Lulworth Beds (Purbeck Beds). In the Purbeck Beds of Dorset and the Weald 39 cycles have been identified among the ostracod faunas which Anderson and Bazley² have termed faunicycles. One faunicycle comprises an alternation of two ostracod assemblages: (1) species identified as tolerant of relatively saline waters (S-phase); (2) species tolerant of brackish to almost freshwater conditions (C-phase). The ostracod genus *Cypridea* dominates the C-phase of the faunicycles. The number of ammonite zones (among other biostratigraphical tools) allocated to the Lulworth Beds (without ammonites) will ultimately depend on the positioning of the internationally agreed base of the Cretaceous period. In southern England the Cinder Bed Member is generally accepted as the base of the Cretaceous although recent work questions some assumptions³⁻⁵. For the calculations here the Cinder Bed faunicycle² is taken as representing the base of the Cretaceous.

If the Lulworth Beds are accepted as representing two ammonite zones, Table 1 shows values for the average length of a faunicycle (based on 20 faunicycles). The assignment of two ammonite zones to the Lulworth Beds may prove to be inaccurate and lead to re-interpretation of the values shown (for example, one ammonite zone for 20 faunicycles would give averages approaching the obliquity figure; M. R. House, personal communication). Anderson and Bazley² considered that if the faunicycles were climatically induced, the precession of the equinoxes might have been responsible.

Sedimentary microrhythms can be recognized in the Lulworth Beds, and evidence of possible annual seasonal rhythmites can be seen in the deposits of restricted pools (Lower Dirt Bed of central Dorset) and also expressed in the pattern of fossil tree-growth rings⁶. There is much sedimentary variation in the Purbeck Beds due to their deposition marginal to a lagoon of fluctuating water level⁷.

The faunal cyclicity of the Purbeck Beds reflects palaeoecological cyclicity that is

not mirrored by lithological microrhythms. Is it coincidence that this cyclicity averages 117 kyr, assuming the Jurassic to be 70 Myr in duration? Is this an expression of the 100-kyr cycle due to orbital forcing⁸? Ostracod assemblages have allowed correlation across north-west Europe in the late Jurassic and early Cretaceous⁹, consisting of groups of ostracod zones and faunicycles. The faunicycles are assemblages of ostracod species; not purely evolutionary sequences, they represent changes in palaeoenvironment and may be laterally variable in their specific content². The fact that the faunicycles persist over rapid lateral and vertical lithological/environmental changes suggests the involvement of some longer-term factors in the cyclicity. Might it be the case that, in palaeoenvironmental settings where sedimentary microrhythms are not manifestly obvious, such as in the marginal-marine Lulworth Beds of Dorset, biotic rhythmicity (ostracod faunicycles) reflects climatic cyclicity due to orbital forcing?

Note added in proof: Anderson¹⁰ has recognized one more faunicycle in the Purbeck Beds. Following assumptions made above the average faunicycle length is 111 kyr.

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Pre-Rhaetic therian mammals

THE discovery of a *Kuehneotherium* tooth in deposits older than the Rhaetic transgression in Somerset, UK, was reported by Fraser, Walkden and Stewart¹, who claim that it is the oldest record of a therian mammal, predating the *kuehneotheriids* from the supposedly 'Rhaetic' locality of Saint-Nicolas-de-Port in eastern France, described by Sigogneau-Russell². However, one of us has recently studied the amphibian and reptile remains collected at Saint-Nicolas-de-Port by G. Wouters and P. Coupatez, and has compared this faunal assemblage with those from better known German localities³. This reveals that the Saint-Nicolas-de-Port fauna is of roughly the same age as that from Kuhn's levels 15-20 of the Halberstadt locality⁴, and should thus be referred to the Norian.

Therefore, the Emborough *Kuehneotherium* and the mammals from Saint-Nicolas-de-Port may actually be of the same age, and may all qualify as the oldest known mammals.

The case of the Saint-Nicolas-de-Port locality illustrates the fact that the Rhaetic stage is of very doubtful value as far as continental faunas are concerned. The Rhaetic was first defined as the top of the marine Triassic in the northern Alps, but even there its validity has been questioned, as its fauna is not significantly different from that of the Norian, so that many stratigraphers have abandoned it altogether⁵. Outside the Alpine domain, the term Rhaetic is even less meaningful, especially in the Germanic domain where correlations are difficult. It seems to be impossible to define a typically Rhaetic vertebrate fauna, and vertebrate localities lumped together in the so-called Rhaetic may be of very different ages. We are therefore in full agreement with the opinion expressed by Fraser, Walkden and Stewart¹ that the Emborough find 'throws doubt on the fundamental distinctiveness of pre-Rhaetic and post-Rhaetic vertebrate faunas'. Continued use of the terms Rhaetic and Rhaetic in a stratigraphical sense causes more problems than it solves, and cannot contribute to a better understanding of the relative stratigraphical position of late Triassic continental vertebrate localities.

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FRASER, Walkden and Stewart¹ have recently announced the discovery of fossil mammal teeth in a terrestrial fissure deposit at Emborough in the Mendip Hills (Somerset, UK). It has been dated by them as 'pre-Rhaetic' following Robinson⁶ who, in the then absence of direct palaeontological dating, invoked comparisons based on rock types, topographical position and the general nature of the faunal assemblage. We disagree with this.

Robinson's use of rock types for dating is inappropriate, since fissure fills and normal stratified sequence are of markedly different origin. The marl lithologies cited by Fraser *et al.*¹ as typical 'pre-Rhaetic' are also known from the 'Upper Rhaetic'³.

We envisage a situation where, in the prolonged period of sub-aerial exposure before, or during, the early Rhaetic, tectonic and solution features formed which

Table 1 Average length of a faunicycle

Duration of Jurassic (Myr)	Duration of each zone (Myr)	Average faunicycle length (kyr)
60	1.00	100
65	1.08	108
70	1.17	117
75	1.25	125

contained little sediment. As sea levels rose during the Rhaetian, these fissures filled with contemporaneous sediment, with additional solution occurring during intermittent periods of local Rhaetian sub-aerial exposure⁴.

When Robinson² dated the Emborough fissure as Norian she was influenced by the absence of mammals, so that their discovery removes this age connotation. Fraser *et al.*¹ distinguish a discrete Norian fauna, in spite of the lack of an independent Norian date for this supposed pre-Rhaetic reptile assemblage, and in disregard of the evidence from the Tytherington fissure⁵ which is dated as Rhaetian using diverse microfloras. Fraser *et al.*¹ dismiss this date, indicating incorrectly that these particular deposits were tectonically disturbed and mixed. Microfloras of Rhaetian age have now been recovered⁶ from six separate fissure fills in Tytherington Quarry and except for one minor slump sequence none of these palynomorph-bearing intervals shows any evidence of tectonic mixing. Whilst some of the Tytherington fissures were tectonically initiated⁶, most show a pre-infill solutional influence or are entirely solutional in origin. In addition to microfloras, five of these fissures also contain fish (including *Pholidophorus*, *Gyrolepis*, *Hybodus*, *Saurichthys*) and crustacea (*Euestheria minuta*) characteristic of the Rhaetian⁶. The microflora and fauna both occur in the same matrix as the supposed pre-Rhaetic Norian reptile assemblage.

Because the Tytherington 'Norian' reptile fauna has been shown to be Rhaetian and because the nature of Robinson's evidence for a pre-Rhaetic age at Emborough is highly equivocal, we suggest that the presence of *Kuehneotherium* could more reasonably indicate the date of the Emborough deposit than vice versa. It is generally accepted that the abundant mammal fossils (including *Kuehneotherium*) from other British fissure fills are of Rhaeto-Liassic age⁷. We therefore place the Emborough deposit in the Upper Rhaetian and see no justification for the date proposed by Fraser *et al.*¹.

We thank Derek Moore for critically reading the manuscript.

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FRASER, WALKDEN AND STEWART
REPLY—We are most interested to learn that on the basis of detailed comparisons

with well-dated German faunas¹, Buffetaut and Martin would now revise downwards the age of the Saint-Nicholas-de-Port vertebrate assemblage. Although there is as yet no other firm evidence to confirm a Norian rather than Rhaetian age for these late Triassic deposits, the presence of *Kuehneotheriids* amongst otherwise acceptable Norian forms does suggest a mammalian occurrence as old as the Emborough *Kuehneotherium* specimens.

As stressed by Warrington *et al.*², the term 'Rhaetic' in Britain is a lithostratigraphic one, not to be confused with the chronostratigraphic stage name 'Rhaetian' which generally extends a little lower. The marine incursion which established characteristic Rhaetic deposits in Britain was a rapid event affecting wide areas almost simultaneously³. It thus provides a useful stratigraphical marker, and it would be difficult to persuade geologists not to use it. Hitherto, all records of late Triassic mammals have post-dated this transgression, but at Emborough, where we have found mammal remains for the first time, the isolated Triassic pocket containing them lies beneath a planation surface associated with Rhaetic (Westbury Formation) sediments. The pocket sediments themselves are completely distinct, and have continental characteristics identical with Norian deposits nearby. We fully agree that in many other places, including parts of mainland Europe, such a distinction could not so clearly be made.

Meanwhile, Whiteside and Marshall claim, on the basis of their own interpretation, that the Emborough deposit in which we have found mammalian teeth is probably of Rhaetian rather than Norian age. Our dating of the deposit followed careful re-assessment in the field as part of a wider study of the fissure deposit phenomenon, and our acceptance of Robinson was arrived at because we found no cause to criticize Robinson's evidence. Certainly, if palynological evidence came to light, the deposit could prove to be Rhaetic, but equally it might be pre-Norian or mid-Jurassic.

The evidence for a pre-Rhaetic age is based on classical stratigraphical argument, reviewed by Whiteside and Marshall, and no evidence to the contrary has emerged. The deposit is in fact closely similar to local Keuper Marl facies and is comparable to pre-Rhaetic boulder and conglomerate deposits elsewhere in the Mendips. It contains no evidence of derived Rhaetic or post-Rhaetic sediments (including the ubiquitous phosphatic basal bone-bed formerly exposed nearby within Emborough Quarry) and is unlike any of the known post-Norian lithologies in the area. If Whiteside and Marshall believe that the deposit belongs to the Upper Rhaetian, then the onus of proof lies with them and we will welcome any new information they can provide.

As regards the dating of the Tythering-

ton deposits, we merely wish to sound a strong note of caution at a locality where the fissures owe their origin to tensional stresses which continued into the Rhaetic. Whether directly attributable to tectonic dilation, or resulting from meteoric re-working or marine flooding of open systems (for example, ref. 4) the effects of remobilization, mixing and contamination can be very subtle. We would not dispute that the fish faunas may be of Rhaetian age, but it would be helpful if the full sedimentological and faunal details of Tytherington were published in order to provide a firmer basis for discussion. However, the age of the Tytherington assemblages has no direct bearing on the age of the Emborough mammal find, and we raised the matter originally because if it does prove to be Rhaetic it further supports our contention (misunderstood by Whiteside and Marshall and by the author of ref. 5) that there is no longer any case for a fundamental distinction between the terrestrial faunas before and after the Rhaetic transgression based on the presence or absence of mammals.

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Flow law for ice in polar ice sheets

DOAKE and Wolff¹ have argued that a relationship between strain rate $\dot{\epsilon}$ and stress τ of the form $\dot{\epsilon} = A\tau^n$ with $n = 1$, rather than the commonly used value of 3 (and where A is the flow law constant), provides a better fit to data from polar ice masses. Weertman² has discussed the implications. I remain unconvinced for the following reasons.

(1) The Devon Ice Cap borehole is within a distance of 3 ice thicknesses from the ice divide. Modelling of flow near divides, with $n = 3$, shows that the shape of the velocity-depth profile changes with distance; tilting data, if analysed by Doake and Wolff's method, would give a value of n increasing from zero near the divide to 3 at about 10 ice thicknesses from it³. Doake and Wolff's analysis is therefore invalid.

(2) At Camp Century, the strain rate measured for $\tau_{xz} = 10$ kPa, where x_L^* and 3 define a rectangular coordinate system, is less than the observational error⁴. The same is almost certainly true at Law Dome.

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Doake and Wolff's diagrams suggest that removal of this point would increase n to ~ 2 in both cases.

(3) The Amery Ice Shelf data lie on the line for $n=3$, not $n=1$.

(4) The measured spreading rate of Ward Hunt Ice Shelf ($\dot{\epsilon} = 3.3 \times 10^{-12} \text{ s}^{-1}$, $\tau = 12 \text{ kPa}$)^{5,6}, not mentioned by Doake and Wolff, lies between the lines for $n=1$ and 3. A significant fraction of this ice shelf consists of old sea ice, so its impurity content will be high. Because soluble impurities 'soften' ice, correction for this would move the data point towards the line for $n=3$. It has been suggested that the ice shelf may be grounded beneath the survey site, in which case the analysis would not apply. I believe that grounding can be ruled out because the surface slope is of the magnitude ($1.6 \times 10^{-3} \text{ rad}$) expected on floating ice⁵, and the slope is towards the Ward Hunt ice rise⁵; it would be in the opposite direction if the site were on the ice rise.

(5) Holdsworth⁷ suggested why the low-stress points from Erebus Ice Tongue deviate from the line $n=3$; crevasses penetrate a significant fraction of the thickness in the outer part and effectively soften the ice. This seems plausible to me.

(6) New data⁸ from Ross Ice Shelf give $n=3.3$.

(7) Surface velocity measurements^{9,10} on ice rises give values of n of 3.13, 3.7 and 4.2 over the stress range 50–130 kPa.

Doake and Wolff believe that borehole tilting is affected by transient creep because flow changes the depth of, and thus the shear stress on, an ice particle. Weertman suggested that this might make the flow law appear linear. This effect should be greatest in the ablation area of a temperate (0°C) glacier; the vertical velocity there is an order of magnitude greater than in polar ice sheets. Moreover, accumulation and ablation change the ice thickness seasonally by about 5 m. However, tilt measurements¹¹ in 12 boreholes in temperate glaciers provide convincing evidence that n lies between 3 and 4.

Measured velocity-depth curves vary widely in shape, even after allowance is made for temperature variations. I believe that differences in longitudinal stresses, which affect the shear strain rate only if the flow law is nonlinear, can account for much of the variation.

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DOAKE AND WOLFF REPLY—Our position regarding the flow law for polar ice¹ (relating strain rate $\dot{\epsilon}$ and stress τ by $\dot{\epsilon} = A\tau^n$) is consistent with Lliboutry and Duval², who comment that "any attempt to determine the creep law of polycrystalline ice is illusory". Because factors such as crystal size and fabric, and impurity content appear to have a major role in determining the deformation of polycrystalline ice, and because these factors cannot, at least at present, be deduced or modelled *a priori*, there seems to be little point in using a complex flow law that requires large but unknown *ad hoc* corrections to be made to fit experimental data. Our approach has been to find the simplest relationship between observed strain rates and deduced stress field that produces the greatest degree of harmony and the lowest range of 'enhancement factors' between different data sets. It is also reassuring to note that Lliboutry and Duval² have independently concluded that, for the Holocene ice at Byrd Station, a linear flow law gives the best fit to field-tilting data.

As to the points raised by Paterson:

(1) Raymond's³ model of flow near ice divides shows that for $n=1$, there is no difference in the velocity profile shape with distance from the divide. An $n=1$ exponent gives a good fit to Paterson's observed velocity-depth profile at Devon Island, while $n=3$ with Raymond's model applied to Devon Island gives a poor fit⁴.

(2) There is common agreement that borehole data are difficult to analyse because of measurement errors and uncertainties. Discarding the point at 10 kPa at Camp Century would in any case lead to a value of $n=1.47$. There is no justification for discarding the point at Law Dome. The measured strain rate (before our adjustment to -28°C) is well above any likely observational error. (The data at Law Dome must in any case be constrained by the requirement of non-negative basal velocity, which led to the two circles (not used in the regression) at the high stress end of our Fig. 2d. Lliboutry and Duval² have produced more detailed arguments about factors affecting flow in boreholes, but we reiterate that if $n=3$ is used, such large variations in A (the flow law constant) are required to fit data that the flow law ceases to have any predictive value.

(3), (4) The analysis by Thomas⁵ of ice shelf data assumed that resistive forces due to shear stress gradients could be ignored. This is not true for either Amery Ice Shelf (where also the site was "situated on a small hill"⁵) or Ward Hunt Ice Shelf (where also grounding may "extend beneath the site"⁵). In reply to the letter by Hattersley-Smith⁶, we do not read Weertman's comments as saying that our data go against a linear creep law.

(5) The only data available from an ice shelf which approaches the ideal

'unbounded' state are those from Erebus Glacier Tongue⁷. It seems unnecessary to postulate a 'softening' of the ice by crevasse penetration, which in any case still does not bring the data to the line $n=3$.

(6) The back-stress pattern⁸ on the Ross Ice Shelf was inferred from an otherwise untested theory of crevasse formation on the underside of ice shelves. Data are sparse and Jezek⁸ has reported discrepancies of up to 1 bar with values of back stress calculated by other methods⁹. Measurements of strain rate and crevasse height were made at different sites, meaning that interpolations had to be made¹⁰. No allowance for temperature variations have been made^{8,10} although this could "represent an approximately threefold variation in the strain rate" (ref. 9) for $n=3$. There is a large scatter in the data of Jezek and others¹⁰, even for the points selected as representing 'best data'. Although this is an intriguing use of unusual experimental data, we do not regard the values as being sufficiently accurate for defining a flow law.

(7) We specifically excluded discussion of ice rise measurements because of the approximate nature of their analysis.

The penultimate paragraph broaches the point that n appears to lie between 3 and 4 for temperate ice. We specifically confined our remarks to polar ice and did not consider temperate ice because of the problem of accounting for free water in the flow law². Because of this water, we see no reason why temperate and polar ice should have similar flow laws.

The final paragraph mentions velocity-depth curves in boreholes. We have shown that if measured temperature profiles are taken into account, better agreement is reached with $n=1$ than $n=3$ in polar ice. There are still discrepancies, which may be due to unmodelled factors such as impurity content or crystal size and orientation; these discrepancies are much reduced for $n=1$. We explicitly allowed for longitudinal stresses in the analyses represented by our equation (1) (ref. 1), but there are no data on their variation with depth. Again, we conclude that on evidence available it is not necessary to use a nonlinear flow law to describe the flow and deformation of polar ice sheets.

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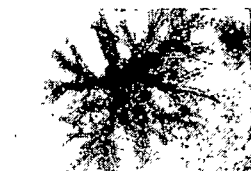
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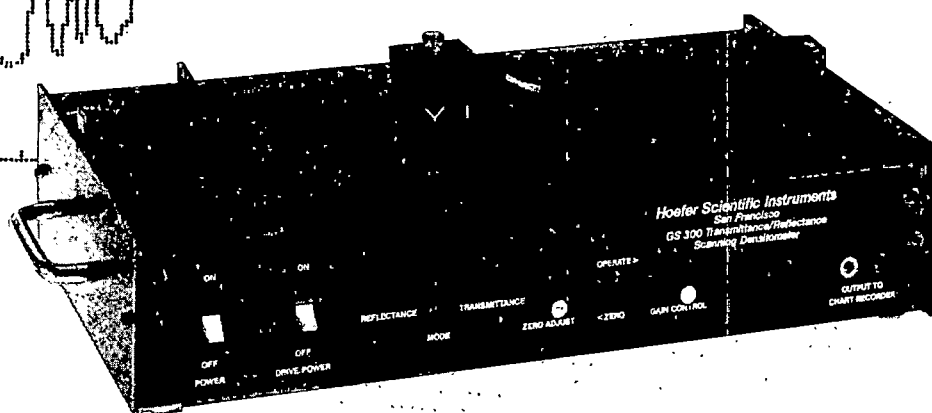
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Cell populations re-examined

from R.F. Jarvis and D. Rossington

Cell biology has been in existence for over 400 years. But only recently has it been possible to study cells in massive numbers.

"... these pores, or cells, were not very deep, but consisted of a great many little boxes ..."

I no sooner discern'd these, which were indeed the first microscopical pores I ever saw, and perhaps that were ever seen ... but methought I had with the discovery of them, presently hinted to me the ... reason of all the Phaenomena of cork; ..."

(Robert Hooke, 1665)

AND so an era began. The basic living unit of plant, animal and man, had been seen and identified. With the Dutch draper Leeuwenhoek's observation, at about the same time, of a central body within salmon blood cells, such observations heralded the beginnings of modern cell biology.

Within the confines of a tiny space (the average mammalian cell is some 15–20 μm in diameter) is a completely automated, computerized factory possessing an organizational capacity to direct the reproduction of cells into masses of tissues with unique tasks, so that a complete body can be built from a single isolated cell.

The living cell may appear fairly simple on the surface but microscopists have long recognized internal structures. The most prominent of these structures, the nucleus, houses the chemical templates of production, the chromosomes. The nucleus is suspended in cytoplasm, a rich fluid containing many complex molecules and many small organelles, and the whole is contained within an outer cell wall, the membrane.

Soon after fertilization the ovum begins to divide continuously to form a mass of

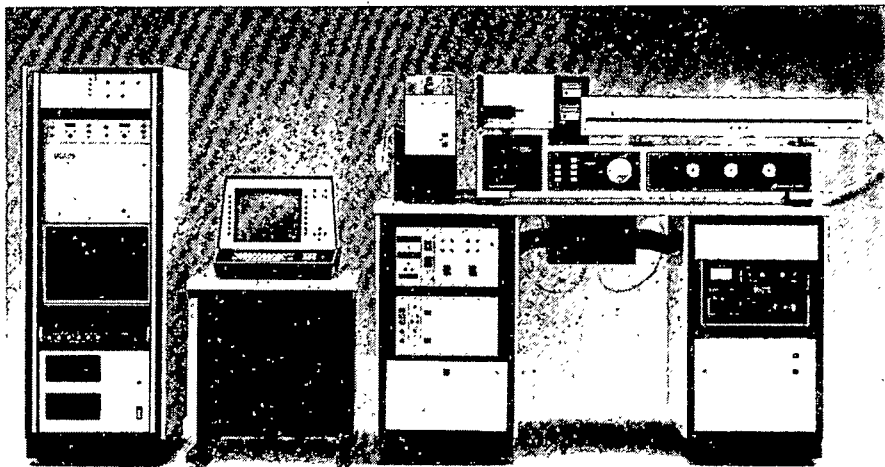


Fig. 2 A typical flow cytometer, using scattered light to determine cell characteristics.

smaller, similar cells. Within this mass of cells, or blastocyst, new cells are produced, and soon the three basic tissues of the fetus — the ectoderm, mesoderm and endoderm — emerge. The layers become entwined and growth continues, with the ectoderm further specializing to produce the skin and nervous system while the liver, pancreas, lungs and other organs, plus the digestive tract, spring from the primitive endoderm. Muscle, blood, bone and the connective tissues will result as the mesoderm divides and specializes.

Depending on the function of the tissue from which a cell is extracted, cells will vary in outer size, shape and internal organization. By studying the morphological and biochemical profiles of the cells, biologists can ascertain the family tissue and, often, whether the cell is healthy.

Methods of examining and classifying cells go back many years. The chemical and physical structures of the cell are routinely viewed after suitable processing to stain the various constituents with a range of organic dyes. Solid tissues are sliced by a sharp knife, or microtome, after the tissue has been held firmly embedded in a wax block or frozen solid. Then, the one-cell-thick section is coated onto a glass slide and stained. Many stains exist to show the presence of the internal structures of the cell and of particular cellular enzymes and both internal and external excretions.

The examination of blood to identify and count the red cells, white cells and platelets has found increasing importance as electrical methods have increased the precision and usefulness of such tests.

Most patients seeing doctors and entering our hospitals routinely have a full examination of their blood to aid in the diagnosis and treatment of their ailments. Apparatus exists to count and size, rapidly and routinely, the main types of blood cells. Blood specimens can be measured at rates of over 100 per hour, and data presented include the haemoglobin content as well as the concentration of several subpopulations of white cells. One such apparatus is shown in Fig. 1.

A new ability to study subpopulations of blood cells and suspensions of dispersed solid tissues has recently been developed, allowing cell surface molecules to be identified. The surfaces of circulating blood cells are not smooth but have many molecules projecting from them. The white blood cells are a vital part of the human immune system that defends us against the challenge of microorganisms which threaten to destroy us in order to live themselves. Although the main types of white blood cells can be routinely distinguished by morphology using manual staining, or automatically counted on a blood cell counter, our understanding of them and hence of the immune system has leapt forward in recent years because of the development of reagents which can identify parts of the cell surface molecules. With these monoclonal antibody reagents it has been possible to divide the white cell population responsible for specific immunity (the lymphocytes) into various subgroups based on the different molecules (or antigens) present on their surface. The complex task of correlating these subgroups according to function and

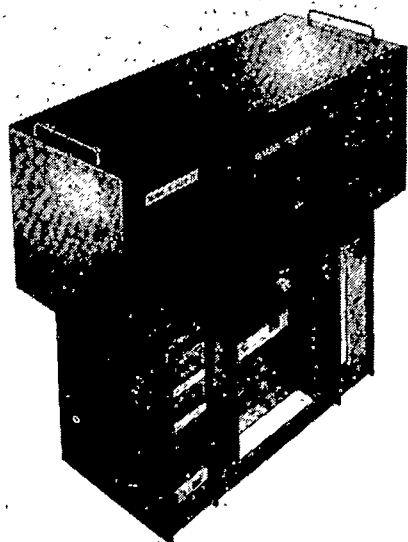


Fig. 1 An automated blood cell counter.

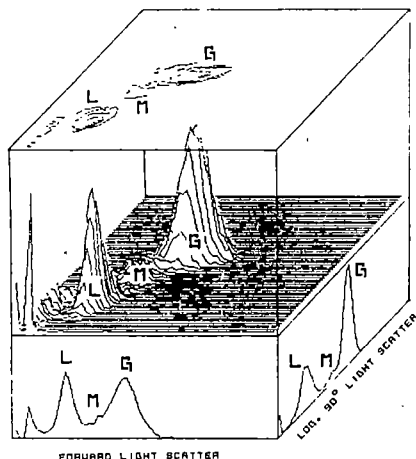


Fig. 3 The distribution of lymphocytes, monocytes and granulocytes displayed from simultaneous measurements of forward angle light scatter and wide angle light scatter as recorded by a flow cytometer, measuring 2,000 cells per second.

role is far from complete but this technique has already led to a better understanding of the various diseases of the immune system.

The circulating blood cells are also studied by haematologists who use commercial monoclonal antibody reagents in the same cell surface techniques to type and count the various immature or mature cells that are present in leukaemia and lymphoma. Here again, the ability to identify subpopulations has led to better diagnosis and monitoring of these tumours of the blood. Modern instruments, called flow cytometers, are now available that will examine many thousands of individual cells each second, and report on their characteristics. A typical flow cytometer is shown in Fig. 2. The cells in suspension are made to follow a confined trajectory through a very intense beam of light. Light is scattered from a cell entering the beam and transmits vital information to sensitive detectors concerning the size and constitution of the cell (as for example, in Fig. 3). Additionally, probes which fluoresce in the bright light can be used to identify particular antigens and other cellular constituents.

New methods in the field of cellular biology will emerge in the future. Pattern recognition computers are already able to evaluate a stained section much as a human does but, at present, are relatively slow. New identification probes will be devised to look inside the cell and evaluate its integrity. Understanding the functions of specialized cells, in relation to those surrounding them, will become increasingly important. The search for knowledge about these basic units of life will go on so that our understanding and treatment of abnormal tissues will continually improve. □

R.F. Jarvis and D. Rossington are at Coulter Electronics Ltd, Northwell Drive, Luton, Bedfordshire LU3 3RH, UK.

Cells in the laboratory

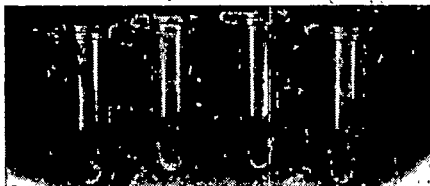
A selection of equipment—some to be seen at the Atlanta meeting of the American Society for Cell Biology.

- Using the direct immunofluorescence staining method, Dako's conjugated rabbit antibody against human IgG (F202) demonstrates the presence of immunoglobulin deposits in the basement membrane zone at the dermal epidermal junction. Positive staining for IgG in certain skin biopsy sites can be a useful tool for distinguishing systemic (SLC) from discoid (DLE) lupus patients. Other helpful applications for this conjugate include the indication of skin disorders such as dermatitis herpetiformis and bullous pemphigoid.

Reader Service No. 100.

- Bioglass reusable microcarrier beads, fabricated from a plastic latex base with a thin layer of glass on the outside are ideal for cell attachment and for easy harvesting. They can be made in a range of specific gravities and sizes for cell culture and viral vaccine applications. Their near neutral buoyancy means easy suspension and slow stirring speeds. Bioglass microcarriers can be cleaned and sterilized like ordinary glass and reused up to 10 times with no degradation of cell growth capacity. European distributors and stockists for Bioglass microcarriers are European Business Associates of Luxembourg.

Reader Service No. 101.



Colourful tubes from Elkay.

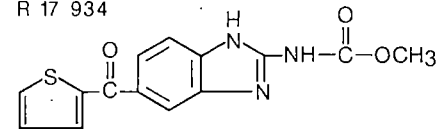
- Elkay's expanded range of 1.5 ml microcentrifuge tubes now offers a choice of two materials and three colours. The range includes 19 different types of microcentrifuge tubes ranging in size from 250 µl to 1.9 ml. Precision moulded from polypropylene or polyethylene, an integral plug cap insures that the tube can be conveniently sealed to eliminate the risk of spillage. This feature also makes the microcentrifuge tube safer for transportation and storage as well as centrifugation. The tubes are available in blue, green and yellow for easy sample identification. These 1.5 ml microcentrifuge tubes can withstand the high centrifugal force generated by modern centrifuges.

Reader Service No. 102.

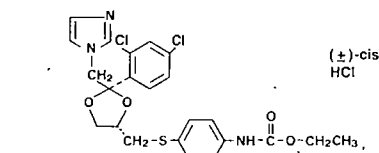
- A new synthetic microtubule inhibitor, R46846, has been synthesized by Janssen Research Laboratories. The *cis* isomer of tubazole-C, this reagent inhibits the rate and extent of rat brain tubulin polymeriza-

tion with an ID_{50} of 3×10^{-7} M—about three times as potent as nocodazole and 12 times as potent as colchicine. The novel antimicrotubule compound R17934 (see below) is also new from Janssen, and as it

R 17 934



(R 46 846)



(±)-*cis*
HCl

is chemically unrelated to the familiar microtubule-disintegrating alkaloids (colchicine, vinca and so on), it should provide a useful alternative in studies of microtubule involvement and function.

Reader Service No. 103.

- The Falcon Labware Division of Becton Dickinson has introduced newly designed tissue culture dishes called Easy Grip. With the Easy Grip feature, the lid and dish can be picked up and stacked easily and cleanly regardless of hand size. The design also keeps the contents undisturbed when removing the lid. Many researchers have claimed the design of conventional dishes makes the handling of the dish a difficult procedure and the contents are occasionally disturbed during the process. The first Falcon tissue culture dish to receive this design is the 35mm size.

Reader Service No. 104.



Getting to grips with the Falcon Easy Grip.

- Oncor Inc. has now developed reagents and protocols for *in situ* hybridization. Any 35 S- or 3 H-labelled probe may be used in this system for the detection of DNA or RNA target in cells or frozen tissue deposited on microscope slides. DNA or RNA targets are detected *in situ* under the microscope after autoradiography. Counter stains such as haematoxylin and eosin and Wrights stain are compatible with the system. All Oncor probes are available labelled with 3 H or 35 S for use with the *in situ* hybridization kit.

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• Now available from Accurate Chemical of Westbury, New York, extracellular matrix (ECM) coating was developed expressly by IBT of Israel for simulating *in vivo* cell growth in an *in vitro* system. As a naturally produced substrate, ECM plays an active role in the control of cell proliferation and differentiation, and should provide greater study potential in cancer and haemostasis research, biopsy specimen growth, and other areas of clinical application. The culture ware is supplied pre-coated with ECM. Also new from Accurate is fibroblast growth factor, a stimulant of growth in tissue culture.

Reader Service No. 106.



Cytometry mechanized on the ACAS-470.

• The ACAS 470 workstation from Meridian Instruments functions as a "no-flow cytometer" that brings methods of automated clonal selection and fluorescence analysis to cell monolayers maintained in a tissue culture environment. This is accomplished through the integration of laser technology, epi-illumination microscopy and rapid, high resolution stage positioning — all under computer control. The intensity of the focused laser beam can be rapidly modulated to provide variable illumination for fluorescence excitation, photobleaching, cell surgery and cell killing. Fluorescent emissions are detected by a photomultiplier and digitized for storage. Results are presented on a colour graphic terminal. Coordinate locations of individual cells can be stored for automated time-based multiple analyses.

Reader Service No. 107.

• Beckman Instruments, Inc.'s Model L7-55 ultracentrifuge combines simple operation, reliable induction drive and built-in diagnostics with an attractive price. The induction-drive ultracentrifuge can run all Beckman high-performance fixed angle, vertical tube and swinging bucket rotors. Model L7 offers speed to 55,000 r.p.m. and forces to 408,000g for everyday applications. It operates at 2° to 20°C with thermistor temperature sensing.

Reader Service No. 108.

• Becton Dickinson has developed a new fluorescence activated cell sorter, the FACStar. This instrument incorporates the FACS cell sorter concept into a new user friendly design. The FACStar analyses cells or other biological particles, at the rate of ten thousand per second, using a laser beam. These cells are characterized on the basis of forward-angle light scatter, 90° light scatter and two-colour fluorescence. Individual cells of interest may then be physically sorted or separated. Operation of the FACStar is computer controlled. Single-key commands allow complicated analysis and sorting procedures to be performed without further operator intervention. The FACStar can also be operated manually so that performance can be quickly optimized without recourse to time-consuming software menus.

Reader Service No. 109.

• The new electron microscope from Philips, the CM 10, is aimed at life scientists requiring advanced instrument control techniques. Central to the CM 10 technology is the Microcontroller, a microprocessor-based microscope control system which links the operator console and the column hardware. It enables control elements to be assigned general functions rather than directly connected to specific lenses or deflection coils. Consequently, each function, such as image focusing, always occurs with the same function knob, regardless of microscope mode or the magnification applied. Operator communication with the Microcontroller is via an interactive screen. Microscope mode and observation conditions are labelled in the screen as are the functions of special CM 10 softkeys, through which both mode and conditions are determined.

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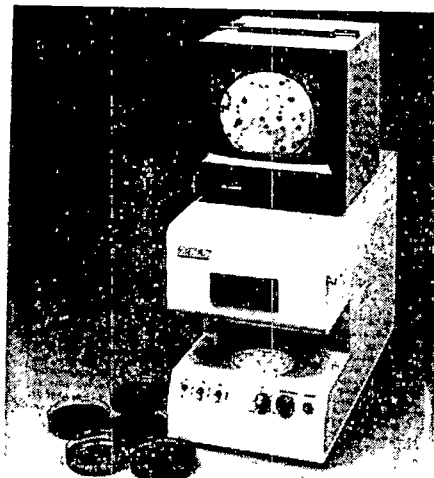
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Reader Service No.36

• A new service from Molecular Biosystems provides custom-modified DNA. Modified oligonucleotides are available at 10–40 base units in length with a non-isotopic label. The modified SNAP oligonucleotides contain thymidine analogues with linker arms attached at C-5; such linker arms terminate in a primary amine or label of choice such as fluorescein, rhodamines or biotin. Amine-derivatized oligomers can be attached to solid supports for immobilization of hybrids. Labelled oligomers can be used for non-isotopic detection of complementary sequences.

Reader Service No. 111.

• Auto-Count, from Artek Systems Corporation, is a new low-cost automatic counter for rapidly counting bacterial colonies. The AutoCount has an adjustable aperture which allows operators to isolate an object or group of objects for separate image counting. Simple adjustment of its size discriminator dial elimin-



Bacterial counting by Artek.

ates objects that are smaller than the determined size threshold. The AutoCount can also be manually adjusted to a proper sensitivity threshold. Every scanned object is flagged with an illuminated dot so the operator can see exactly which objects have been included in the count. Objects can be virtually any shape, clearly defined or hazy, dark or light. Artek is a subsidiary of Dynatech Corporation and the Auto-Count is available from Dynatech in the United Kingdom.

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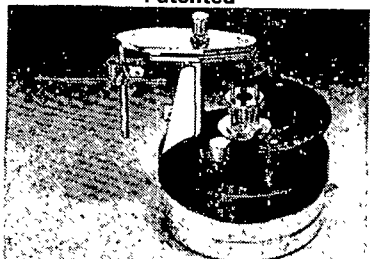
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Reader Service No.48

ATCC
Catalogue of
Cell Lines & Hybridomas
5th edition, 1985

ATCC's catalogue now available from ATCC, 12301 Parklawn Drive, Rockville, MD.

• The fifth edition of the ATCC Catalogue of Cell Lines and Hybridomas has just been published, describing over 2,650 lines. The 305-page catalogue provides data on human, animal and plant cell lines; details on more than 200 hybridomas; a listing of cell lines involved in patenting; recommended media and growth conditions for many cell lines; and formulae for standard cell culture media and reagents. Lines are indexed by species, tissue cell type, tumour type, monoclonal antibody specificity, special applications (including virus studies) and originator's designation. Copies of the catalogue are available free of charge in the United States. Shipments outside the US will be charged \$6.50. ATCC is exhibiting its range at the meeting of the American Society for Cell Biology, Atlanta.

Reader Service No. 113.

• The Coulter D10 Th is a semiautomatic diluter designed to provide dilution of platelet rich plasma (PRP), for use with the Coulter Thrombocounter. The D10 Th can be operated in any one of the three modes which all control the same dilution function. By pressing the black touchplate on the front of the instrument, by utilizing the optional pipettor, or by the convenient foot switch. All three operating modes enable the D10 Th to aspirate, and then precisely dilute 3.3 µl PRP sample in 10 ml of Isoton 11, all in eight seconds.

Reader Service No. 114.

• Ricin A and B chains are now available from ICN Biochemicals. Whole ricin is a highly toxic protein of molecular weight 66,000 and is isolated from castor beans. The A chain is the enzymatically active subunit which interferes with protein synthesis, and the B chain binds to carbohydrate moieties and mediates entry into the cell. The A chain is virtually devoid of any toxic effects for whole cells as long as it is not attached to the B chain of ricin through sulphide bonding. The ricin A chain has been bound to monoclonal antibodies, hormones and numerous other cell surface ligands. Applications of conjugated ricin A include histological cell identification and removal of unwanted normal cells from suspensions.

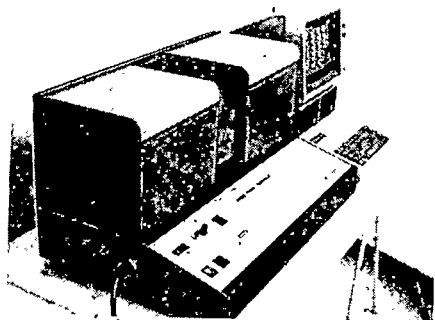
Reader Service No. 115.

Non-radioactive DNA label

The Orogenics DNA Chemiprobe kit was incorrectly described as a radioactive labelling system in *Nature* of 12 September. In fact the innovation is that Chemiprobe is a non-radioactive system for DNA labelling. For more details on Chemiprobe write in number 99 on the Reader Service Card.

• Bioscreen is a new automatic and compact analyser system from Labsystems. Using vertical photometry, the system measures microorganism growth. Practical applications are possible in many fields: food and beverages, cosmetics and toiletries, pharmaceuticals, water analysis, industrial effluents monitoring and basic biological and toxicological research. Bioscreen is fully automatic with a capacity for 200 simultaneous individual tests. Each testing cuvette is only 400 µl, therefore decreasing reagent consumption. And, the Bioscreen liquid dispensing system is easily autoclavable. Bioscreen consists of an analyser unit, a desk-top computer with customized software programs, a printer and disposables. The analysing unit itself contains a dispenser/diluter and an incubator connected to a photometer. The system sets on most any laboratory counter. During the test process, the computer reads information such as the initial cell population size, cell growth, growth speed and cell generations.

Reader Service No. 116.



Polaron's latest microcomputer-controlled tissue processor.

• Polaron Equipment's new E9200 automatic tissue processor employs a bench-top microcomputer for system control, instead of the electromechanical card reader previously used. The use of the computer provides much more flexibility and is much easier to operate than the older system. The reagent delivery system incorporates eight separate reagent storage bottles, each with its own peristaltic pump. The E9200 handles all reagents from initial osmication to 100% resin embedding and is capable of processing up to 126 samples at one time.

Reader Service No. 117.

• A new product data sheet from Schleicher & Schuell gives specifications for its Uniflo sterile, disposable laboratory syringe filters. Made with low protein-binding, pure cellulose acetate membranes, the 25-mm Uniflo filter offers high sample recovery, no extractables, and a process volume of up to 150 ml for materials as viscous as media with 10% bovine serum albumin. For each of the four available pore sizes (0.2, 0.45, 0.8, and 1.2 μm), a specifications chart gives flow rate, pressure rating, average hold-up volume, and application areas.

Reader Service No. 118.

• Nalge Company, a division of Sybron Corporation, has available a free wall chart to simplify the selection of the right filter for the job. This new four-colour chart includes complete selection and applications data for reusable filter holders and presterilized, disposable filter units, syringe filters and membranes. The chart also includes selection information for Nalgene 25-mm syringe filters, 47-mm membranes, and reusable holders.

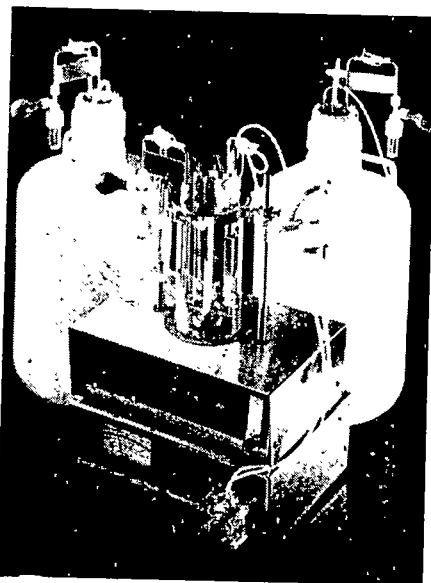
Reader Service No. 119.

• Bellco Biotechnology's new modular magnetic stirring system, the Sci/ERA quad drive, provides precision control of cell growth parameters for up to four remote stirring units from a single master controller. The one-to-nine position stirrer modules accept spinner flasks up to eight litres in size permitting convenient system expansion. Remote units are connected to the master controller via 10-foot heavy duty cables with locking plugs, allowing ample room to pass into or through incubators. System options include a sealed gel-electrolyte battery to protect cell cultures against power loss.

Reader Service No. 120.

• The PSS-80 automatic peptide synthesis system from Applied Protein Technologies Inc. features a unique in-line reaction monitoring system, fifteen randomly accessible, refrigerated amino acid reservoirs and a negative pressure delivery system. APT's Custom Peptide Synthesis Service furnishes peptides promptly in quantities of 10 mg to 10 grams or more. All are sequenced to demonstrate their purity and integrity. APT's proprietary chemicals include PEP-TIES which are insoluble solid-supports for immobilizing peptides and proteins. For polypeptide isolation and purification, PEP-SEPS are amino acid specific immobilized reagents. PEP-SEPS facilitate the design of DNA probes by isolating specific peptides or peptides containing specific amino acids. APT products can be seen on Booth 326 at the Atlanta Cell Biology meeting.

Reader Service No. 121.



Available from Techmation, the new VirTis chemostat.

• The VirTis Omni-Culture Chemostat bench-top fermentation system for continuous culture experimentation and aerobic/anaerobic studies is now available from Techmation. The system is designed to provide accurate, reproducible data, facilitating scale-up; it also regulates temperature, agitation, filtered air or gas flow and the transfer of medium. To maintain sterility throughout prolonged research studies, all components coming into contact with fermentation liquids are made of materials that are non-toxic, non-corrosive and repeatedly autoclavable.

Reader Service No. 122.

• A new vibration isolated bench from Photon Control of Cambridge is designed for electrophysiologists, neurophysiologists, cell biologists and molecular biologists using precision micromanipulators and probes of micrometre or sub-micrometre geometry. Users of the Model BT Series of biotechnology benches claim an ability to routinely investigate small cells (5 μm radii) with longer impalement times, and a substantial improvement in

signal to noise ratios. The BTT bench top is constructed from synthetic granite which consists of 96% solid granite aggregates bonded together, the remaining 4% is a special well damped matrix material.

Reader Service No. 123.

• The Skatron Microwash I and II 96-Well Automatic Microplate Washers have been designed for both enzyme immunoassay and radioimmunoassay applications; the versatile Microwash II has additional facilities which make it suitable for use with cell monolayers. The microplates are washed by selecting one of the three wash programs which permit either 3, 4 or 5 repetitions of the wash/aspirate cycle. The rinse program is used to prevent blockages caused by the build-up of crystalline deposits by flushing the wash liquid from the instrument at the end of the working day.

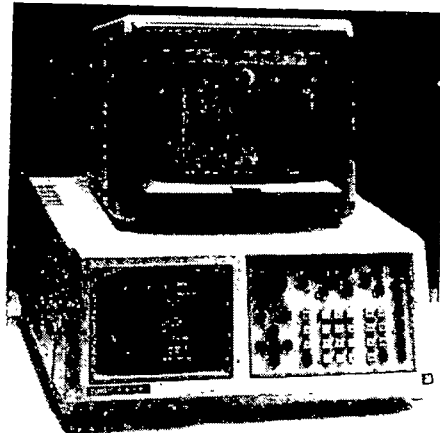
Reader Service No. 124.

• A new data sheet on the Kontes Micro Ultrasonic Cell Disrupter is now available. Designed to be capable of disrupting samples in a reproducible manner, the disrupter's LED output indicator provides a precise measure of the load imposed by a sample; previously documented responses can be confirmed to be re-occurring. Tip displacement amplitude is maintained by Kontes' monitor circuitry which assures stability by continuously correcting amplitude variations.

Reader Service No. 125.

• Optomax Inc. has introduced two new image analysis systems. System V is an automatic system utilizing dual microprocessors and high-speed measuring circuitry. Provision of circular and rectangular frames, variable in size and position, plus user-drawn frames, allows areas of interest within the image to be defined. In addition to a single detector, System V features a twin 'grey slice' detector and also a programmable 256 grey level auto detector. Vids V is a high resolution semi-automated system utilizing a graphics tablet and IBM PC or compatible computer. Input from the TV camera is viewed on the display monitor and features to be measured are 'drawn round' or 'dotted' using a hand-held cursor.

Reader Service No. 126.



A compact Optomax image analyzer.

A seller's market for graduates

from Richard Pearson

Against the trend in other sectors, the demand for new graduates in scientific and technical subjects in Britain is strong.

EARLY last year the *Employment* column forecast that a revival in the job prospects for new qualifying graduates was imminent. In the event the upturn in recruitment has been faster and more widespread than anticipated, with employers from sectors as diverse as oil and chemicals, manufacturing, retailing and financial services all reporting growing numbers of vacancies. While the high fliers and information technology specialists have been in particular demand, graduates in the pure sciences and the applied sciences have also been beneficiaries. In 1984 the number gaining employment in industry and commerce was already 25 per cent higher than during the labour market doldrums of 1980–81 and is set to increase again in 1985. The one no-growth area is for jobs in higher education itself.

The International Labour Office (ILO) has recently highlighted the booming UK graduate labour market, and reports that it is not mirrored in most other countries. In the United States over half of new PhDs cannot find the type of employment for which they were trained. Unemployment among chemists and chemical engineers, for instance, was at its highest level for a decade in 1983. In West Germany, the number of jobless university graduates has quadrupled during the past four years to a total of over 115,000. Teachers, engineers, natural scientists, lawyers and economists were particularly hit. In France there were nearly 60,000 members of managerial and supervisory staff without jobs in early 1984, while in Switzerland, joblessness among young university degree holders has risen from 2 to 5 per cent in recent years — much faster than the general unemployment rate. Japan offered a mixed picture. Job offers for 1984 university graduates increased by 4.7 per cent as compared to 1983 thanks to a new phase of industrial innovation combined with an economic upturn; however,

openings for high school graduates were expected to decline by 12.9 per cent.

A common thread everywhere is that women graduates face more difficulties than men in finding and keeping a professional job, partly because a larger proportion of women take degrees in less "marketable" subjects, but also due to sex discrimination. In addition, the ILO report concludes, more and more professional workers are being forced to step down on the employment ladder and accept jobs for which they are overqualified, setting off a chain reaction which affects the entire labour market.

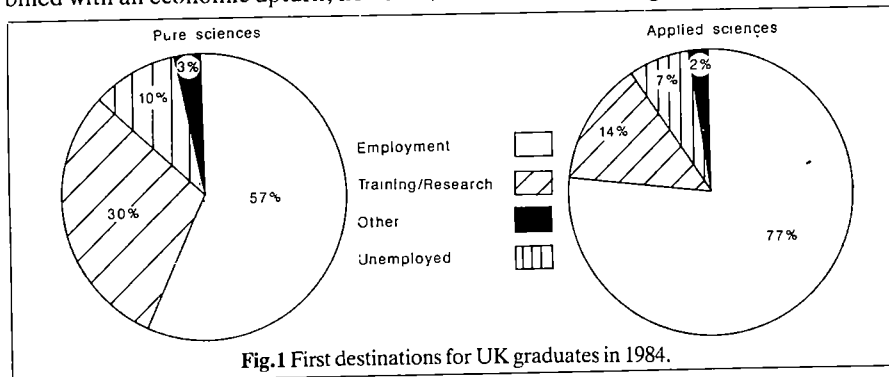
The final figures for the United Kingdom in 1984 show that of the 30,000 graduating in science subjects from the universities, nearly two-thirds went into employment, 15 per cent went onto further research and about 6 per cent went into some form of vocational training, the latter percentage having continued to fall sharply in recent years. Of particular note are the declining numbers entering teacher training; since 1980 the number has fallen by nearly one-third, and totalled just over 1,100 in 1984, of whom less than half were mathematicians or physicists. This means that the already serious shortages of mathematics and physics teachers is likely to get worse over the next decade. About one in eleven scientists were still seeking work six months after graduation. Of those scientists entering permanent employment, over half went into manufacturing industry, and about 14 per cent, a steadily declining fraction, entered the public sector. Less than four per cent got jobs in the education section while twice as many went into accountancy. In terms of actual jobs, the most common destinations were management services, and research and development and associated support activities. The pure scientists were more likely to go into further research or training, and also had higher

unemployment rates than the applied scientists, while more of the latter went directly into employment (Fig.1). Women were more likely to enter teaching than their male counterparts. The data for the polytechnics to be published shortly are expected to show broadly similar patterns.

On the salaries front the average for those starting jobs in autumn 1984 was about £7,000 per annum, with many companies in the growth sectors of oil and chemicals, electronics and information technology offering nearer £8,000 per annum. Salaries for 1985 are expected to be about £500 higher, with a few of the top payers in central London offering starting salaries in excess of £9,000 per annum as they seek to attract the "best" talent. Post-graduates with relevant degrees or experience relevant to the job being filled can command substantially higher salaries; most, however, receive salaries little higher than first degree graduates.

In the scramble to attract the "best" people, where, in the case of non-technical jobs employers often judge personal criteria before academic criteria, the traditional gentlemanly calm of the "milk round" is becoming outdated. In past years, virtually all the companies visiting colleges did so in the spring term, apart from the accountancy companies who were able to plead special circumstances and start their recruitment in the autumn term. Nowadays more companies start their recruitment campaigns before Christmas to try and catch the best students, and some are offering previously unheard of inducements such as "signing-on fees" if jobs are accepted quickly.

At the same time many students are becoming more confident of their job prospects and are concentrating on their examinations during their final year. They are delaying job search until after they have qualified, sometimes then taking a break of several months. In this way the graduate recruitment round, formerly concentrated into the spring term, is starting earlier, and extending later. If the anticipated growth in demand for graduates is matched by the expected fall in the numbers graduating then graduates who are well qualified, in both personal and academic terms, will be able to dictate not only the type of job they enter but also the time of year they seek work. □



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How to tell success from failure

Next week's summit will have served its purpose if the participants merely agree that they should meet again. Some popular goals are unattainable, others deserve more attention than they will get.

Two people who have never previously laid eyes on each other will spend Tuesday and Wednesday of next week in each other's company under the most trying circumstances. Of late, President Ronald Reagan and Mr Mikhail Gorbachev have spent much of their energy in mutual public recrimination, which would be understandable against the background of the antagonism of the two governments they respectively lead, but which is hardly calculated to make for a cosy chat at Geneva. To make things worse, they have arranged to meet without knowing just what they will talk about if, as seems likely, they run out of things to say about their prime topic of conversation, arms control. Yet, as if to ensure that their social embarrassment will haunt them for years to come, the two leaders have encouraged people everywhere to expect great things from their encounter. *Pravda*, not to mention the *New York Times*, has for weeks had space for almost nothing else. This is one reason that the meeting is bound to seem a disappointment, a measure of the gap between people's expectations and reality.

Luckily, none of this implies that the meeting will be a waste of everybody's time. Those who expect wonders from Geneva should be content if the two participants agree only that they will meet again within the year. (Exactly a year from now would inconveniently coincide with the mid-term election season in the United States.) That alone would wonderfully repay the cost of all those airline tickets to Geneva. Less than that would be worrying for everybody. So an agreement to meet again is the least that must be looked for. What follows is a check-list of plusses and minuses by which departures of next week's meeting from that minimum should be judged.

Arms control

That arms control should be central to the agenda is natural, although the widespread belief that this is the sole touchstone of success is mistaken. Most of the recrimination of the past few weeks has centred on the US plan to build a defence against ballistic missiles, the Strategic Defense Initiative or SDI. The Soviet Union says that SDI is a provocation, the United States that a programme (not a plan) to make people everywhere safe from the threat of annihilation could not but be a boon, and that in any case the programme consists only of research; it could become a plan only a few presidential terms from now. Both sides are on solid ground, as in most confrontations born of conflicting interests. From the visit to Moscow earlier this month of Mr George Schulz, the US Secretary of State, it is clear that there will be little give or take on this issue at Geneva. The worst case will be that in which one or other of the participants digs in his heels on SDI, so as provoke a summit meeting as indistinguishable from a shouting match as that in Vienna in 1961, when the late Mr Nikita Khrushchev could not control his temper, read the still-new President John F. Kennedy a demeaning lesson and laid the ground for the Cuban missile crisis almost exactly a year later. The best hope is that next week's actors will perform a little more imaginatively.

A few ingredients could realistically be added to the minimum agreement to meet again within the year. In the field of arms control, there is no reason why the two sides should not agree in principle that there should be a treaty to prohibit either of them from destroying the other's Earth satellites. The Soviet Union

has been asking for such a treaty for the best part of a year, but the United States has replied by saying that it is at a disadvantage at this stage in the evolution of these esoteric weapons. Both perceptions may be correct, but the technology of anti-satellite (ASAT) weapons is at such a rudimentary stage that it would be wiser to live with an imbalance than to embark on the development. Thus an agreement in principle (the details would have to be worked out) to negotiate an ASAT treaty could take the edge off next week's disappointment, and would be worth having in itself.

ABM treaty

Another piece of frosting on next week's otherwise disappointing cake would be an agreement, again in principle, that the Anti-Ballistic Missile (ABM) Treaty of 1972, signed when the notion of *détente* seemed set in concrete, should now be reaffirmed and renegotiated. There are two problems. So much SDI talk has seemed to threaten the ABM treaty that reaffirmation is necessary, but the ambiguities that have allowed the US State Department and the Pentagon to disagree about the testing of SDI components need to be resolved. The treaty is ambiguous because its drafters did not know about SDI, but letting it lapse would be a setback. If SDI is indeed only a research programme, reaffirmation would cost nothing. Optimistic realists will be looking for one or other (or, preferably, both) of these refinements in next week's communique. The ground is not nearly well enough prepared for agreement to be reached next week, but a declaration of principle would allow the negotiators to get down to work. But all of this would be extra to the bare minimum agreement.

Familiar components of other arms control agendas will be absent from next week's agreement. Although the Soviet Union has been operating a unilateral moratorium on the testing of nuclear weapons (due to expire at the end of the month), there is no chance that the superpowers will agree next week to move decisively towards a comprehensive test-ban treaty. The United States is at present at a military disadvantage in being lumbered with the MX missile system, and will have a need to test warheads for whatever succeeds as the mainstay of its strategic force; giving up testing now would not be popular in Washington, but both sides are increasingly discomfited by the knowledge that even if there were a test-ban, France and China would stay outside. Difficulties about remote verification also persist, although less seriously than when the last round of serious negotiations began in 1977, but these might well be overcome by more liberal arrangements for *in situ* monitoring and on-site inspection. It would cost Mr Reagan and Mr Gorbachev nothing to agree that the draft treaty of November 1979 should be dusted off to see whether it will suit present circumstances.

But what of the issues central to the negotiations on strategic arms that have been under way at Geneva since the beginning of the year? The high expectations of next week's meeting stem from the enthusiasm that has been endangered for a substantial reduction of nuclear weapons. The two sides have indeed put forward proposals that outwardly differ very little from each other. The general idea is that strategic nuclear forces should be reduced roughly by a half. The Soviet proposal would selectively reduce present nuclear forces, the United States would limit

warheads instead. The obvious difficulty is that the viability of such comprehensive mutual restraint depends crucially on the details of an agreement, not its general outlines. The most that can be expected next week is that the two governments will acknowledge that the gap between them is not huge, and that they will then wish their negotiators at Geneva good luck. That, obviously, would be a plus. The corresponding minus is the danger that the Soviet Union will link the continuation of these potentially fruitful negotiations with a demand that SDI should be abandoned. That could spell a further deterioration of East-West relations, with possibly dreadful consequences. The question would be more rationally discussed when the draft of a treaty on strategic arms limitation exists, which is another argument for a second meeting.

What else?

The upshot is that there can be no firm agreement on arms control next week, but at best a series of agreements to agree. If there is to be a second meeting, the participants should therefore arrange that they will have other things to talk about. But what? Mr Gorbachev has been saying that arms control must be central to next week's talks, but that overlooks the truth that arms control is feasible only when political relationships are secure. President Reagan, trying to broaden next week's agenda, has been advocating (at the UN General Assembly) a partial list of the world's trouble-spots on which both Afghanistan and the Middle East are conspicuous, the second by its absence. In reality, such an agenda would cause more trouble than emollience. In the early 1970s, it proved possible to trade the promise of Soviet friendliness for that of US technology, particularly (as it turned out) the products of US farm technology now accumulating in the silos of the Middle West. No such easy accommodation is now possible. Nor is there much mileage in the almost ritual negotiation of improved scientific collaboration, the standby of politicians in need of something on which to strike agreement.

But why should not the United States and the Soviet Union negotiate some of the practical issues that divide them? The West's embargo on advanced technology, administered by COCOM, would actually be more coherent if the Soviet Union were given a chance to comment on the nonsenses that frequently arise. The endless troubles about spying in the past year could have been avoided if there were a general understanding about the line dividing the tolerable from the unacceptable. And the time will come when the two superpowers must talk about the political framework for the relationships between European states, East and West. It will be an uncovenanted plus next week if, having agreed to meet again, the two strangers agree to broaden future agendas in this way. □

Education in a mess

A new wave of anxiety about US science education may, this time, bring action.

SCIENCE and engineering education in the United States has frequently been held up elsewhere as a shining example to be followed. In Britain, for example, the many studies over the past two years focused on the shortage of scientific and technical skills have ritually applauded what has been done in the United States. But if US science education at the undergraduate level has the appearance of ruddy health, it is only skin deep. The system, if it can be called that, is in trouble. Science education is facing its biggest crisis since the panic that gripped the United States in the early 1960s, after the Soviet Union beat it into space with Sputnik.

It is true that in the United States undergraduate institutions seem to have adapted more quickly than those elsewhere to the booming demand for skills that have anything to do with computers, thanks largely to the recognition by many US science-based businesses of where their interests lie. (It also helps that the tax system is so weighted in favour of corporate giving that it would

be public relations suicide for a major corporation not to be seen to give away a few millions of dollars every year or so.) But there is more to life, and science, than desk-top computers. The problems are many, and are now being spelled out in painful detail to the National Science Board, the policymaking arm of the National Science Foundation. The board, to its credit, has established a committee (inevitably called a task force) to examine the problem and to see what the foundation should do about it. There is mounting and disturbing evidence that the quality of teaching in both public and private universities is declining. Faculty are ageing and are not being replaced at a sufficient rate, especially in engineering. Laboratory instrumentation is, despite corporate munificence, seriously out of date, particularly in institutions not famed as research establishments. And increasing teaching loads all too often force universities and colleges to rely on the teaching of undergraduates on new graduates, for many of whom English is not a first language.

Quantity is another and a daunting aspect of the problem. The proportion of young people in the United States going on to higher education in science and engineering is only a half of what it is in Japan, while there is mounting evidence that demand is being constrained both by the high cost of higher education and the continuing poverty of high-school education at all but the excellent institutions. Can the United States continue to be astonished at the imbalance of its trade with Japan?

Who is to blame for this mess? The National Science Foundation. No other federal agency can be expected to do the job. Since the late 1960s, the proportion of the foundation's budget devoted to science and engineering education has declined from something close to 30 per cent to rather less than 5 per cent. The foundation finally gave up pretending to take seriously its statutory obligation to education at the undergraduate level in 1981, when its paymasters in the Office of Management and Budget (backed by the new White House) snuffed out all but one of its activities in that area. The one remaining programme provides a niggardly \$5 million a year for laboratory equipment in non-research institutions.

The result of this neglect is that university teaching has come to seem a chore to faculty members at many institutions, not an activity vital to the function of an institution. (There are some honourable exceptions, chiefly among the private universities.) Research, by contrast, brings its own rewards, both intellectual and (mainly in the United States) financial. What could the National Science Foundation do to help? Plenty. It could make awards, symbolic and monetary, for the preparation and dissemination of teaching materials, preferably making full use of the still largely untapped potential of computers. It could arrange (and pay for) national meetings and teaching workshops where good teachers would describe their techniques. It could make special grants to graduate students who display real talent for teaching. It could even expand its teaching equipment programme to include the research universities. Most important, it could renew its commitment to the notion that in an advanced democracy some comprehension of science, even by those who will not later become professional scientists, is a necessity, not a luxury.

A credible programme for science and engineering education at the undergraduate level would provide what is at present lacking, a subset of university teachers whose primary commitment would be to excellence in education. The amount of money needed is not large by US standards: \$50 million would be a useful start, increasing to perhaps twice that amount over a few years. Fortunately, all the indications are, the colossal deficit notwithstanding, that Congress is sympathetic to the idea and is willing to give the science foundation a shove in the right direction: it has demanded a plan of action by next March. Happily, the task force's study will be completed before then, in January, and not before time. By any analysis, the strength of the United States rests on its scientific and technical workforce, as do all of its hopes for the future. There is no known alternative to diligent study and excellent teachers. Americans should not need to be told that. □

Indian technology

Quest for self-reliance runs into trouble

New Delhi

SCIENTIFIC research in India is being steered by Prime Minister Rajiv Gandhi in a new direction intended to take the country into the twenty-first century — fast. Gandhi has made it clear that his government will not tolerate “rubbish science” or inefficient industry, and that all research must be productive. Indian scientists have been told not to waste time in reinventing technologies that can be purchased, but to work on those that others are not willing to sell. His adviser Mr L. K. Jha has gone a step further in suggesting that industries should not hesitate to import not only technologies but, if necessary, whole factories as well.

The plans for the overhaul of research and development and liberalized import policy stem from Gandhi's conviction that Indian scientists have been pampered for too long without being accountable and that industries have enjoyed protection for years at the cost of efficiency. Scientists are now being asked to deliver and industries to compete and catch up. “India had been pushing hard on science while neglecting the technology aspect”, Gandhi told a recent press conference. “Our research and development must be such that it really ends up with a finished product.” Gandhi feels that India's 750 million people are entitled to a better deal from Indian technologists. Since they have failed, there should be no objection to importing foreign technology.

The Council of Scientific and Industrial Research (CSIR) is the first agency to be asked to streamline its research. Other ministries that preside over research establishments will follow. Gandhi has directed CSIR to concentrate on a few key areas or “technology missions . . . instead of frittering away energy on a whole gamut of fields.” Following Gandhi's directive, CSIR director general Dr S. Varadarajan has undertaken the massive exercise of revamping the research activities of the council's 40 or so institutes. Several CSIR projects that lack definite goals are to be axed and pilot plants that have failed to generate interest in industry may be scrapped. About half of CSIR research is related to developing “import substitutes”. Now that import is freely allowed, such projects will face closure. Basic research will be a casualty. As far as CSIR is concerned, the only basic research permitted is that relating to “frontier areas” such as biotechnology or microelectronics.

Not everybody in CSIR is happy about the shape of things to come. “Our Prime Minister wants us to leapfrog to the

twenty-first century”, says a senior scientist. “But we cannot get there without mastering twentieth century technology.” Although a month has passed since the review began, few technology “missions” have been identified. “We can easily close down unproductive projects”, says Varadarajan, “but it is not easy to replace them with new ones.” CSIR has about 20,000 scientists and technicians, and it is an open question how to relocate those whose projects are to be axed. In the past, Indian industry has been more or less forced to adopt CSIR technology. According to one CSIR director, requests for processes from his laboratory dropped from 77 in 1984 to four in 1985. It is feared that with industries free to import the best from abroad there will be no more takers for CSIR knowhow.

The liberalized policy on the import of technology has also come under attack from sections of the scientific community inside and outside CSIR, as is shown by recent controversies over the move to import silicon and fibre optics technology. Critics believe the import policy is a negation of self-reliance, the hallmark of Indian science during the days of Jawaharlal Nehru and Indira Gandhi. “Indian research and development has been castrated”, says Baldev Singh, former chief of the technology utilization division of CSIR. “The country's laboratories appear almost like condemned institutions”, says Mr P. S. Deodhar of the Electronics Trade and Technology Development Corporation. There is a fear that industries based on indigenous technologies will be wiped out and that CSIR will face a slow death.

The fear is not unjustified. A cottage industry making silicon-carbide crucibles, and a factory that just started making “chlorosilane” using CSIR technology are facing closure as these products can now be freely imported. CSIR spent eight years developing SWAT-106, a chemical that improves oil flow through pipelines. But now four multinationals are planning to bring in their own technologies for a flow improver.

The *Hindustan Times* in a leading article said: “More and more public undertakings are hankering after foreign deals little bothering to find out whether appropriate indigenous knowhow exists. What is the idea of having big scientific establishments if their talents cannot be utilized locally?”

In the electronics sector, the clamour for foreign collaboration is at its peak. According to Deodhar, the import policy is luring businessmen “whose motive is commercial rather than technical”. Consumer electronics items with international

brand names are being marketed by Indian companies that have sprung up overnight. Some 29 giant computer companies from abroad are planning to enter the market, virtually sealing the fate of the state-owned Electronics Corporation of India Limited.

According to Prem Shanker Jha, a noted economist, the present import policy has spawned literally hundreds of “screwdriver units” in the electronics and automobile industries, “the two potentially biggest customers of capital goods industries”. There are three foreign collaborations for passenger cars, 10 for commercial vehicles and 14 for scooters and motor cycles. The government-owned Maruti car factory has rejected an Indian consortium's offer to set up an engine production line in favour of a Japanese company. Britain is setting up a plant near Bombay that will make 12,000 electric vehicles a year, preempting the electric vehicle project of the public sector Bharat Heavy Electricals Limited (BHEL), which is itself purchasing anticorrosion technology from a US company, ignoring processes developed by a CSIR institute.

“From one extreme of self-reliance, the pendulum has swung to the other”, says Baldev Singh, “and many of the current imports in the name of high-tech are really irrelevant to taking India into the twenty-first century.” Meanwhile, the number of foreign collaborations shot up from 440 in 1984 to 752 in the first six months of 1985 and foreign exchange reserves dipped from £4,800 million in April to £480 million by the end of July.

The peculiar situation in Indian science today is that heads of scientific agencies who privately worry about the technology import policy publicly remain silent or defend it. Many agree that the 1982 technology policy statement (TPS), which warned against indiscriminate import, has now been more or less torn to pieces. But Professor M. G. K. Menon, planning commission member and architect of TPS, will not comment on the new policy. And Rajiv Gandhi's government still swears by TPS. “The government has not deviated from the concept of economic self-reliance and the liberalized policy of import will not throttle indigenous research or industry”, the Prime Minister recently told newsmen. But sceptics, including the president of the Indian National Science Academy Dr C. N. R. Rao, who strongly opposed import of silicon technology, still believe that liberalized imports will stifle indigenous research.

Professor Abdul Rahman, a former CSIR director and science policy specialist, has warned that modernization cannot be achieved with imports of technology. “This”, he said, “has been amply demonstrated in Latin America which is saddled with debt, and in Iran where the Shah's hurry to modernize with massive technology inputs led only to political crisis.”

K. S. Jayaraman

Biotechnology

Diluted optimism at Osaka

Osaka

THE city of Osaka made its bid last week to establish itself as the Japanese "capital of biotechnology" with what was billed as "the first international conference on biotechnology". While that may be something of an exaggeration, the conference, thanks to generous support from the regional government, must have been about the first to hear experts from the Soviet Union describe their national biotechnology programmes alongside executives from many of the major biotechnology companies — Cetus, Calgene, Cytogen, Biogen, BioEurope and the like.

Within Japan, the Kansai region (which includes Osaka, Kyoto and Kobe) can fairly claim to have taken the lead in traditional rivalry with Kanto (the Tokyo-Yokohama megalopolis). Many of Japan's biotechnology companies have their research laboratories in the region, while Kyoto and Osaka University dominate basic research in molecular biology.

The state of international rivalry is harder to assess. Although Japan and the United States often regard themselves as the only players in the game, the European Communities representative was keen to scotch the view that Europe is not an active commercializer of new ideas. It was pointed out that most of the new (if still few) products of biotechnology were first produced in Europe: monoclonal pregnancy test kits (the Netherlands, 1980), biochemically produced human insulin (Denmark, 1982), recombinant amylase (Denmark, 1982) and recombinant α -interferon (Austria, 1985). Europe is also far ahead of the rest of the world in building new biotechnology research centres and providing integrated courses of study in the universities.

The UK outlook was more gloomy. Some praise was given to the establishment of new companies such as Celltech with "intellectual assets from the public sector and financial assets from the private". But there was a warning that lack of funds in Britain had pushed basic research to the brink of an irreversible decline; there may soon be nothing new to commercialize.

Japan's biotechnology industries are seen as building a better base for the long-term future. At present, commercial emphasis in the United States and Europe is on the production of new pharmaceuticals. That is where, in the relatively near future, completely new products, and new sources of profit, can be created. But it is in plant agriculture and food processing that the really huge returns on investment can be expected towards the end of the century. A very wide spread of companies in Japan has set up biotechnology research laboratories and knowledge of biotechnology is diffused through all kinds of in-

dustry. This diversity may pay dividends.

The Japanese tradition of focusing on production technology, rather than on an exotic end-product, is also at work in biotechnology. There is massive investment in exploiting biotechnology to improve existing processes — better bioreactors, enzymes, lipids and surfactants.

That biotechnology research is concentrated in large companies may also work in Japan's favour. A couple of years ago, Japan was bemoaning its lack of new venture capital while the United States was celebrating the host of innovative small companies into which the stock market was pouring money. But now there is a feeling that new venture businesses will not be big enough to capitalize properly

on their innovative achievements.

Not everyone was worried about international competition. The high level of cross-licensing agreements that has characterized the first phase of the growth of biotechnology, together with the diversity of uses to which biotechnology may be put, was seen by some as ruling out the kind of national rivalries found in electronics. But representatives from the South-East Asian countries were pessimistic. Dependent as many of them are on agricultural production, they stand to benefit most from biotechnology. But they have neither the facilities nor the manpower for large-scale research; recent developments, such as the successful patenting of new types of seeds, have them worried. The low attendance at lectures given by South-East Asian representatives must have helped confirm their fears.

Alun Anderson

Computerspeak

Hope for international standards

Washington

THE world is one year away from agreeing fundamental standards for controlling the communication of computers, according to the leader of the British delegation at a crucial set of meetings held during the past two weeks in North America. The negotiations involve more than half a dozen countries whose governments have been the most active since 1977 in attempting to arrive at a set of standards for inter-computer communications. The 300 or more delegates are here to discuss the latest development in Open Systems Interconnection (OSI).

The delegates are members of the sub-committees from the Geneva-based International Organisation for Standardisation and represent trade associations and government institutions. The main political impetus has come from the United States, Britain, Japan, West Germany, France, Canada and Scandinavia. The OSI meetings require the countries involved to move towards agreement on a comprehensive package of standards in seven distinct areas. These include the disciplines on how computers should be connected to telecommunications circuits, how those data can be stored and displayed, how one network can communicate with another to allow the "global addressing of computers", how errors in data in the network can be detected and corrected, how messages can be synchronized and their delivery controlled. The other standards enable types of data to be identified as they are transferred between computers over national boundaries and between different computer systems.

The sessions have been taking place in Toronto, Gaithersburg (Maryland), Syracuse (New York) and Durham (North Carolina).

Bryan Wood, leader of the UK delega-

tion, says "there is increasing support for a single standard independent of manufacturer". That indeed is the wish of the British government, which has refused to allow the UK telecommunications authority British Telecom to join with IBM to operate a data management network on the grounds that such a partnership would stifle competing systems and allow IBM's own standards to dominate. The IBM communications standard, recognized as a possible rival to OSI, is called System Network Architecture (SNA), and is still being developed in parallel with OSI.

The Europeans and Americans in particular hope that SNA will not hamper OSI development but that it will be used for intercommunications between IBM computers and that the OSI standard will be used for any other communication. The seven levels of the OSI standards, which are at various stages of ratification, are also meant to accommodate communications from any closed network to another.

Computers connected to each other within a factory or office complex would be examples of closed loops, called local area networks (LANs). Such networks will be able to be connected to each other using the OSI standards. They will be able to communicate with each other over an interconnecting network, possibly an international one, whatever the standard used at the local end. The Ethernet system originally devised by Rank Xerox and the recently announced IBM token ring, both forms of LAN, will be accommodated by the OSI standards, claim the delegates to the American conferences.

Bill Johnstone

Bill Johnstone, on sabbatical from his post as technology correspondent of The Times of London, will be writing on technology subjects for Nature during the next few weeks.

Eureka

Optimism in France . . .

"EXTREMELY satisfactory." That is how French research minister Hubert Curien described the results of the second ministerial conference on Eureka, the French-inspired initiative to develop new European high technology products, in Hannover last week.

The meeting decided on 10 projects, seven of which involve French participation. French officials were impressed that "the people who said no" to government finance of Eureka (which decoded means the British) are now saying "perhaps". Sir Geoffrey Howe, British Foreign Secretary, announced that British companies involved in Eureka projects could apply to the Department of Trade and Industry's £250 million annual "Support for Innovation Scheme", and said that he would not place an upper limit on the proportion of that fund that might be devoted to Eureka.

The French view, however, is in reality little different from the British. President François Mitterrand earlier this year announced that FF1,000 million would be devoted to Eureka in 1986, but it is now clear that none of this is new money, additional to the existing government research and development budget. According to Yves Sillard, the president of the French oceans and fisheries research council IFREMER and the man also responsible for the French Eureka projects, there will be no strictly new money in 1986. But more money could be provided later. As M. Hubert Curien, research minister, put it, there is now a line in the French research budget for Eureka, and this is politically defensible. M. Roland Dumas, foreign minister, stressed that the primary object of Eureka is to get new European technology on the market, thus emphasizing market pull rather than technology push—a point of view on Eureka adopted very early on by Britain.

According to Curien, the principal objective at Hannover was to make a list of projects, the first concrete plans, and that has now been achieved. With ten settled, fifteen more wait in the wings while detailed agreements are reached, and some 300 potential projects were on the table at Hannover, with many of them still in the running. "These are good days for Europe", Curien said.

These are, however, still honeymoon days for Eureka. No country or company has yet felt the pain of rejecting its own product in favour of one developed by outside companies under the Eureka label, which is what the logic of Eureka will ultimately demand. "There may be competing products even within Eureka", Curien added. "But we don't want the competition to lead to our losing the race with the United States or Japan."

According to Curien, Eureka will give

what would otherwise be merely a few bilateral or trilateral projects greater scale and greater speed than they would have outside Eureka. Eureka is accelerating what might have happened anyway, Curien said.

The controversial question of a secretariat for Eureka is to be decided, and the secretariat established, by 31 January next, but it should be small and "light", Curien said. Its location is not yet agreed, with countries divided roughly between those who support Brussels and a loose

connection with the European Commission, and those who do not. The French position is that the Commission's work and research should be treated as "complementary" to that of Eureka—which would argue, perhaps, for a Brussels base but not Commission domination of the Eureka secretariat.

Could multinationals be involved in Eureka projects? Yes, according to the French, if they had a base in Europe with independent power of decision. The role of countries beyond the 18 nations present at the Hannover meeting was not discussed, said Curien. Collaboration would not be ruled out but "Our object is clear. It is Europe, Europe." **Robert Walgate**

Eureka

But who is going to pay?

Hannover

In the eyes of the West German host delegation, the Eureka conference was a limited success. Both foreign minister Hans-Dietrich Genscher and research minister Heinz Riesenhuber praised the second Eureka conference as a "starting point for a progressive Europe".

Nothing of real substance was actually decided, it is admitted, but the 36 foreign and research ministers at least left with good intentions. For example, all were agreed that the third Eureka conference should take place next year in London. Following traditional EEC practice, discussion of the most urgent problems has been put off until then. The ministers also decided in principle to create a "small and flexible Eureka secretariat"—Britain and West Germany eventually agreeing to this French proposal. West German Chancellor Helmut Kohl and France's President François Mitterrand are backing Strasbourg as a location for the secretariat but there is also support for Brussels.

All participants agreed that Eureka projects should only serve civil purposes—in stark contrast to the original plans of the French, formulated after their refusal to take part in the US Strategic Defense Initiative (SDI). West Germany rejected all military aspects knowing that only civil purposes of the planned research would find support in this country, although like Britain and Belgium, Germany intends to participate in both Eureka and SDI.

The ten projects most favoured in the initial list include all three suggested by West Germany. The ten are: high-power lasers for material processing; a computer network for European scientific research; investigation of atmospheric pollution independent of national borders; amorphous silicon for production of electricity from solar energy; superfast vector calculators; robots for the textile industry; biotechnology; optoelectronic production systems; diagnosis of sexual diseases, in particular AIDS; European standards for home and school computers.

But there is still a cloud hanging over Eureka—there was no agreement about how to finance these ambitious projects. France made a gesture of offering to put FF 1,000 million toward Eureka projects: the West German government has not yet decided on the level of its support, preferring industrial companies to initiate and finance the projects.

Meanwhile criticism has come from some circles within German industry. If the industry is to finance the projects, why is it the politicians who meet to select them? The president of the German Trade and Industry Association, Wolf von Amerongen, went as far as saying that industry has absolutely no use for Eureka. The government disagrees. **Jurgen Neffe**

Refusniks appeal

LEONID Ozernoi, the Soviet Jewish astrophysicist who three years ago went on a protest fast coinciding with the eighteenth General Assembly of the International Astronomical Union (IAU) now seems to have lost faith in hunger strikes. In a message to the nineteenth General Assembly of IAU, which opens in New Delhi next week, Ozernoi says that his 1982 fast, and a similar, collective fast the following year with other refusniks seeking to emigrate to Israel had "disappeared without trace, like a black hole, in the depths of the Soviet emigration offices".

He adds that thanks to the support of friends in the Soviet Union and abroad, he has managed to stay out of prison or mental hospital and has not lost his job. But "nobody can be sure".

Ozernoi asks the participants at the IAU meeting to "raise [their] voices in protest against the retention of the refusnik scientists in the Soviet Union". To those who consider such appeals "inappropriate in the fight for peace", he says that silence will be interpreted as tacit support for Soviet attitudes towards the refusniks.

Vera Rich

Plant genes

Rifkin opens new campaign

Washington

JEREMY Rifkin, the campaigner against genetic engineering, has taken up a new cause: protection of plant germ plasm. In a suit filed in a federal court last week against the US Department of Agriculture (USDA), Rifkin, whose legal manoeuvres have held up field trials of genetically engineered microorganisms for two years, accuses USDA of "gross negligence" in looking after its collection of seed samples at Ft Collins, Colorado. The suit says the National Seed Storage Laboratory (NSSL) is so poorly managed and underfinanced that "thousands" of samples collected from all over the world are lost each year.

The Rifkin suit is the latest wrinkle in a highly politicized international dispute over the control of germ plasm. NSSL says it always makes available its samples (excluding narcotics) in response to a request from a qualified plant breeder, regardless of the request's country of origin. But Rifkin and his fellow plaintiffs, who include pressure groups from Malaysia, Spain and Canada, object to seed companies using the NSSL collection to develop usable breeds that may be highly profitable.

USDA drags feet

Washington

CONGRESSIONAL concern over the US Department of Agriculture (USDA)'s slow preparations to regulate biotechnology research is unlikely to be eased by a report published last week by the General Accounting Office (GAO). GAO found that in 1984 and 1985 together, the department will support biotechnology to the tune of \$40.5 million, including 87 projects that involve planned releases of genetically engineered organisms into the environment, most in the next five years. But GAO says that USDA was unable to provide information on the number, location and objectives of specific biotechnology research projects.

Denied official information, GAO conducted a questionnaire survey of institutions conducting USDA-supported research. The results of the researchers' own estimates of the risk of their planned environmental releases are, however, unlikely to surprise anyone. Asked whether their planned environmental releases would represent a reason for concern, 75 of the 87 contemplating such experiments checked the "no problem" box, nine the "very minor problem" box and three did not know. Asked what effort would be required to correct any problem that did arise, one solitary brave soul said a problem might be uncorrectable, noting, however, that this was the least likely outcome.

Tim Beardsley

The Rifkin version of this is that "industrialized nationals and transnational corporations attempt to seize control over the world's genetic resources in order to exert unchallenged power over the world's economy in the Genetic Age". The reason for the international involvement in a suit is that NSSL was for a time the principal repository of the seed collection maintained by the International Board of Plant Genetic Resources (IBPGR), an organization funded by the World Bank and the United Nations Food and Agriculture Organization (FAO) which has catalogued some 300,000 food crop varieties from around the world.

Rifkin says that NSSL, which is a back-up safety repository, has haphazard collection policies; that most food crops other than a few staples are not collected at all; that samples are inadequately documented; and that genetic variation in the samples is lost because seeds are planted out too infrequently and in unsuitable locations. Rifkin wants USDA's total budget for germ plasm increased from its current level of about \$12 million to \$40 million, the figure recommended in an internal USDA study and in a study by the seed company Pioneer Hybrid.

While rejecting some of Rifkin's specific charges, USDA officials say they accept that the suit has been brought in good faith and readily agree that the situation at NSSL is far from satisfactory. Dr Charles Murphy, of USDA's Agricultural Research Service, admits that lack of storage space is becoming "a serious problem"; NSSL has put in a bid for extra funds towards a new storage building to replace one that will soon be full. And Murphy agrees that NSSL's lack of knowledge about many of the samples it has received is its "biggest concern"; efforts are now being made to implement a new computerized database management system. But the figures Rifkin quotes for the proportion of unusable samples make "no sense", according to Murphy. Many samples have not yet received "plant identification" documentation simply because NSSL has not yet been able to ensure the stocks are viable.

Dr Lewis Bass, curator of NSSL, also says that germ plasm storage should be a "higher priority programme", as it "will have far more impact on mankind than anything in the space programme". He says NSSL could use more money but that "we do a good job with what we get". Nevertheless, he agrees that the process called "enhancement" — getting the useful properties into strong varieties that can be used by breeders — has barely started.

IBPGR officials decline to comment on the specific charges being levelled at NSSL; they say that it is only by maintaining a strictly apolitical stance that IBPGR

has been able in the past to deliver samples to any country in the world.

The international aspect of the row is likely to come to a head this week at an FAO meeting in Rome. There, IBPGR's cherished apolitical stance may be seriously threatened: radical Third World countries have demanded that IBPGR be given a statutory foundation and guidelines that would make patenting of seed varieties illegal. In order to avoid becoming politicized, IBPGR may have no choice but to duck out of FAO's sphere of operations and become an independent scientific body.

Tim Beardsley

Bioengineering

Plan for field tests of bacteria

Washington

THE first environmental releases of genetically engineered organisms are expected to be approved this week by separate US government agencies. The Environmental Protection Agency (EPA) will give the nod to Advanced Genetic Sciences of Oakland, California, to conduct a field test of a bacterium modified to protect from frost damage, while the National Institutes of Health (NIH) will formally approve a proposal by Agracetus of Wisconsin to field-test a modified tobacco plant as a model for studying plant viral disease resistance.

Advanced Genetic Sciences had voluntarily sought approval for its experiments from NIH, but withdrew the application after legal complications bogged down a similar proposal from Stephen Lindow of the University of California at Berkeley.

The environmental impact assessment for the Agracetus proposal was signed by NIH director James Wyngaarden in September; NIH officials have in the meantime been deciding how much of the assessment will be made publicly available. The decision is that the entire document will be released, with only the details of the test location deleted. But it is unlikely that NIH will become involved in future environmental impact assessments of agricultural field trials; under new rules recently adopted, NIH can leave regulation to another agency if this is considered appropriate, and EPA is likely to deal with most such proposals in future.

Advanced Genetic Sciences has produced a modified strain of *Pseudomonas syringae* which has had deleted from its genome the gene for an ice-nucleator protein which in natural *Pseudomonas* is expressed in the cell membrane and triggers the growth of ice crystals when the temperature drops below freezing. Experiments with *P. fluorescens* will follow. Tests will involve treating strawberry plants with the modified bacteria.

Tim Beardsley

Israel

Medicine confronts Jewish law

Rehovot

TENSION between Israeli medicine and Jewish law, as embodied in the Torah, is growing. The immediate difficulty is that the practice of autopsies and transplants is being inhibited by the provisions of Jewish religious law that the body of a dead person must be treated with the utmost respect, must be buried quickly and in its entirety, and must "not be exploited".

Dr Mordechai Halperin, the physician and ordained rabbi who heads the Schlesinger Institute for Medical-Legal Research at the Orthodox-oriented Sha'arei Zedek Hospital in Jerusalem, says that one consequence is that autopsies cannot be carried out unless there is a "reasonable and immediate" prospect of the saving of human life.

During the past few years, the numbers of autopsies carried out at Israeli hospitals have declined sharply, chiefly as a result of the Anatomy and Pathology Law of 1981, a result of pressure from the religious parties, which requires that an autopsy requires not only the authorization of three

physicians (as of old) but also the approval of the next of kin, and which allows the procedure to be blocked by almost any relative, even a distant cousin. At Halperin's own hospital, autopsies are now few and far between, perhaps fewer than a dozen a year. But he says that there is no necessary conflict between medicine and Jewish law, and that autopsies are no longer necessary for the training of medical students or even for confirming physicians' diagnoses. At Sha'arei Zedek, where full-scale autopsies require the consent of the hospital rabbi as well as that of a senior physician, post-mortem biopsies are quite common.

Elsewhere, the proportion of hospital deaths followed by post-mortem examinations has fallen to 10 per cent, but there are even fewer in Orthodox Jerusalem. Professor Haim Lichtig, a pathologist at the Haifa Technion medical school, says that students did not participate in a single autopsy last term, and that textbooks, films and the examination of preserved organs are a poor substitute for the "the real thing".

Pathologists such as Dr Nina Hurwitz of the Kaplan Hospital also argue that autopsies remain a valuable means of "quality control" in the practice of medicine, and that post-mortem biopsies or non-invasive diagnostic techniques such as CAT-scanning are inadequate alternatives; the discrepancy between clinical and pathological diagnosis has remained obdurately unchanged for 40 years, she says.

The availability of organs for transplantation is similarly constrained. Halperin says that Jewish law would treat as "murder" the removal of organs from a body whose heart was beating but whose EEG record was flat on the grounds that people had been known to recover from brain death. The removal of livers for transplantation would be permissible because artificial organs are not available, but kidney transplants are not always justifiable because kidney dialysis is possible. Halperin points out that life expectancy after kidney transplantation is not very different from that with dialysis.

The strict application of the law is not always accepted. One paediatrician who is religious considers autopsies necessary and has performed them himself. On kidney transplants, he suggests that one kidney should be removed from a dying person who may still be maintained by a respirator. Even some members of the Orthodox establishment appear to recognize that changes are required. Former Chief Rabbi Shlomo Goren, who also served as Chief Chaplain to the Israeli Army, is reported to have been furious that skin for transplanting to injured Israeli soldiers has had to be imported from overseas.

Nechemia Meyers

New RI director



PROFESSOR John M. Thomas, at present professor and head of the department of physical chemistry at the University of Cambridge, will become director, resident professor and director of the Davy Faraday Research Laboratory in the Royal Institution, London, on 1 October 1986, in succession to Sir George Porter, who has been nominated as president of the Royal Society.

French agriculture

More help for farm lobby

THE French government has taken further steps to strengthen the research base of its largest industry, agriculture. The ministers of agriculture, M. Henri Nallet, and of research, M. Hubert Curien, have announced a new programme to double government assistance to private research projects. This follows the earlier announcement that the agricultural research council (INRA) will be reorganizing its research programme so as to adapt more quickly to the opportunities in biotechnology (see *Nature* 24 October, p.660).

The stimulus for the new programme, which will take government support for private research to FF110 million a year, was apparently the discovery that agricultural and food companies spend only 0.25 per cent of their turnover on research.

Interestingly, the new assistance programme will focus on the downstream part of agriculture. The four principal themes are nutrition and toxicology, product quality, fermentation and food engineering. INSERM is also to pay more attention to the epidemiology of nutrition, while there is to be a global strategy of research on food and health.

The emphasis on food safety seems to derive in part from the recognition that if the European Communities manage to establish a central scheme for food and drug regulation, along the lines of the US Food and Drug Administration, French food exports could be jeopardized. But the government seems also to be anticipating a weakening of European price support for food and other produce and the more competitive market that would then come into being.

Robert Walgate

Academies go west

Washington

THE US National Academy of Sciences is going west. Together with the National Academy of Engineering, it has been given \$20 million by the Arnold and Mabel Beckman Foundation to establish a 50,000 square foot west-coast building complex consisting of an auditorium and meeting rooms at Irvine, California. The Irvine Company has donated a seven-acre site adjacent to the Irvine campus of the University of California, worth \$6 million.

Arnold Beckman, now 85, is the chairman and founder of Beckman Instruments Inc., of Fullerton, California, and has recently made multimillion-dollar donations to both of his former colleges, California Institute of Technology and the University of Illinois (see *Nature* 17 October, p.568). The gift to the national academies includes an endowment to maintain and staff the building, which will be known as the Arnold and Mabel Beckman Center.

Beckman pointed out that California now leads the nation in memberships of both national academies. During fiscal year 1985, the academies and related institutions held more than 2,000 meetings in their east-coast buildings in Washington DC and Woods Hole, Massachusetts, but only 70 meetings were held west of Colorado. The new building will be used to support the full range of the academies' activities; Beckman has, however, expressed a particular desire that it should be used for the study of social and ethical issues arising from developments in science and engineering.

Tim Beardsley

UNESCO

Soviet move too late?

THE United Kingdom, which has given notice to, withdraw from the United Nations Educational, Scientific and Cultural Organisation (UNESCO) by the end of the year, last week found an unusual ally in the Soviet Union, in the final days of UNESCO's Sofia conference. British delegates return home this week after a series of post-conference committee meetings and present ministers with recommendations for Britain's final decision.

UNESCO reaches its 40th birthday this month, but whether the battered organization will survive much longer has been called into question by the departure of the United States, along with 25 per cent of the organization's budget, at the end of last year. If Britain follows suit in December, as is likely, UNESCO will lose a further £5 million from its annual budget.

Timothy Raison, Minister for Overseas Development, spelled out Britain's worries at the beginning of the conference last month. The main concerns are that UNESCO is too top-heavy, bureaucratic, poorly managed and politicized, particularly in areas of disarmament policy (duplicating the work of other UN organizations) and government control of communications in the developing countries. Britain also considers that UNESCO should demand zero real growth in next year's budget and not increase contributions required of member states following US withdrawal. Specific proposals were presented to the conference as conditions for continued British membership.

Although several concessions were made to Britain in Sofia and the meeting was considered exceptionally harmonious by UNESCO standards, none of the major changes demanded by Britain were adopted. This always seemed unlikely; UNESCO's long-term strategy, the reason for convening the conference, was decided by its secretariat in Paris well before delegates arrived in Sofia — an example, in fact, of the centralized control that Britain is seeking to change.

A major stumbling block to the Western countries' demands for UNESCO's financial responsibility and an end to political patronage is acknowledged to be Amadou-Mahtar M'Bow, the director-general, a skilled manipulator, strongly supported by the developing countries. Hence last week's surprise development, in which the Soviet Union and its allies said that they would remove their support for M'Bow at the end of his term in 1987.

Whether this move will encourage the United States to open negotiations to rejoin UNESCO remains in doubt. And the events at Sofia may well not be enough to cause a last-minute change of heart amongst British ministers. **Maxine Clarke**

Satellite broadcasting

Direct service standards tested

Washington

A RIGOROUS and novel series of tests of the technical attributes of three different broadcasting systems has just been completed at the Lewis Research Centre in Cleveland, Ohio, under the supervision of the National Aeronautics and Space Administration (NASA) by the US interests in satellite television. The tests will be crucial in assisting the United States to adopt soon a technical standard for its satellites.

The tests were conducted by communications specialists and untrained observers under the auspices of the Direct Broadcasting by Satellite Association (DBSA, which represents most of the parties involved in the new industry). DBSA will present a summary of its findings and recommendations by the end of the year to the Federal Communications Commission (FCC), which in turn will give its ruling on technical standards to the four operators who now have approval for DBS satellite construction. The ruling will be made next spring at the earliest.

The Lewis tests were comprehensive. They began in the middle of September and were concluded two weeks ago. The three systems under test were examined in four principal areas — video (picture), audio, teletext (information display) and scrambling (coding signal). Experts and untrained observers gave subjective views on the quality of television pictures resulting from each system.

The systems tested were two forms of NTSC, the method now used in the United States in terrestrial broadcasting, and a version of MAC (multiplex analogue component), the system favoured, in a different form, by the United Kingdom. The results will play a significant part in determining the technical composi-

tion of the US DBS satellites due for launch in late 1988 or early 1989.

Several companies have expressed an interest in operating DBS television services in the United States since FCC invited applications for slots in the geosynchronous orbit. In June 1983 a sectional meeting of the World Administrative Radio Conference (WARC), the body internationally responsible for assigning broadcasting frequencies and satellite positions, allocated eight satellite slots to the United States.

There are at present four approved DBS systems — Satellite Television (a subsidiary of Comsat), United States Satellite Broadcasting, Dominion Video Satellite and Hughes Communications Galaxy. A further six applications for DBS services are being considered. If all are approved, the technical system adopted by the DBS operators for transmission must be able to prevent satellites from interfering with one another. Some will be using the same frequencies, but will have been separated in different slots, while others will be clustered in the same orbit but operating on different frequencies, as each slot can carry 32 channels.

All four approved operators plan multichannel systems of six to 10 channels. One aspect of the tests is to find out the degree to which each signal format is susceptible to particular interference.

The scrambling tests are also of vital importance as the DBS services are likely to be supported by subscription and may need to be protected from piracy. The Lewis tests checked whether signals could be received and viewed by pirates despite the protecting code and whether there was any deterioration in the signal quality received by the legitimate viewer because of the code's presence.

Bill Johnstone

Brenner to quit MRC post

BRITAIN'S top job in molecular biology will become vacant in 1987, when Dr Sydney Brenner plans to step down as director of the Medical Research Council's Laboratory of Molecular Biology at Cambridge. Brenner said last week that he plans to leave the laboratory at the age of 60, so as to be able to spend more time on his own research. He hopes to find himself a base elsewhere in Cambridge, and a small research group with which to pursue his present interests.

Brenner has been director of the MRC's best-known laboratory since 1979. An exile from South Africa by origin, Brenner joined the laboratory in 1957, when it was merely a barely tolerated collection of huts in a courtyard of the Cavendish Laboratory. During these early years, he worked with Francis Crick on the series of experiments that demonstrated that the genetic

code is a triplet code, and contributed substantially to the then rapidly deepening understanding of messenger RNA. More recently, Brenner has succeeded in opening up the study of the nematode *Caenorhabditis elegans*.

Brenner's spell as director of the Cambridge laboratory has been clouded by the consequences of a serious road accident in 1979, when a leg suffered a compound fracture. His colleagues marvel that his energy has been so little impaired.

The consequences of the vacancy at Cambridge will be considered by MRC at its meeting later in the month. The secretary, Sir James Gowans, said last week that all concerned are aware of the importance of the appointment, and that Brenner's successor will be sought on the international market. □

Climatic change with nuclear war

SIR—In August 1984, I pointed out in *Nature*¹ that the article by Turco and others² on nuclear winter did not arrive at a firm scientific conclusion, and is subject to numerous doubts. Fourteen months later, one of the authors, Carl Sagan³, moderated his statement, which had been reiterated in publications as diverse as *Parade*⁴ and *Foreign Affairs*⁵, that nuclear winter is a robust conclusion. His acknowledgement that the question must be further investigated is welcome. Unfortunately, the current article³ contains ambiguous and inaccurate statements.

As an example, Sagan³ quotes from the recent analysis by the International Council of Scientific Unions⁶. He fails to mention the conclusions of this study: that no nuclear winter is to be expected in the Southern Hemisphere, that considerable temperature declines could occur only in the northern latitudes in the summer and

the mid-continental areas. In mid-summer the effect will not be worse than those in fall or early winter. By contrast, the original TTAPS report, which Sagan calls a "valid first-order assessment", predicted a minimum hemispheric average temperature of -23°C .

To escape the danger of nuclear winter, Sagan suggested the reduction of stockpiles, which unfortunately requires faith in the reliability of Soviet compliance. Sagan has published in *Foreign Affairs*⁵

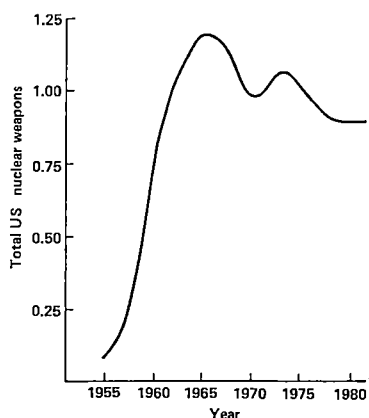


Fig. 2 The history of the relative numbers of US nuclear warheads. There has been a clear reduction in the total numbers. The scale is relative, with 1972 = 1.00.

data on the past and future numbers of nuclear weapons in the various stockpiles (Fig. 1). By contrast, the US government published⁷ relative figures on the time dependence of the American stockpile in regard to number of explosives and in regard to megatons (Figs 2, 3). It will be noted that the former have declined in the past two decades by 30 per cent, the latter by 75 per cent. This latter value is particularly significant in predicting worldwide effects. All of this was independent of disarmament negotiations.

To diminish the likelihood of the catastrophic impact of warfare, one should give most serious consideration to defensive measures. These may not only deter war but, in the horrible case of actual hostilities, the effect of nuclear war would be substantially reduced.

A basis of Sagan's claims is the work published by Alvarez and colleagues^{8,9} which adduces the presence of an iridium layer deposited 65 million years ago as evidence that an asteroid several miles in diameter hit the Earth. The subsequent dust excluded sunlight and led to the extinction of the saurians and many other species. On 11 October 1985, an additional paper was published¹⁰ where deposition of a terrestrial carbon layer was found to coincide with the iridium layer. The authors offer the plausible explanation that approximately 10^{30} erg was set free by a collision with an asteroid. This killed much of the vegetation, set forest fires and

gave rise to soot, the darkening of the sky and to the consequent biological catastrophe.

The survival of approximately half the living genera witnesses the toughness of life on Earth. A reasonable estimate of both the energy released and the smoke

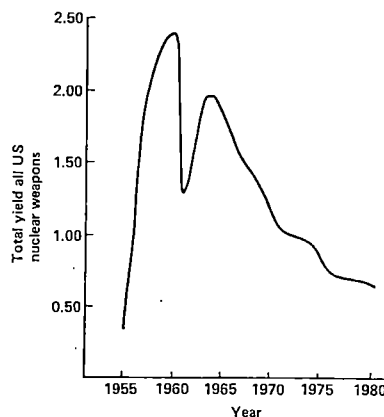


Fig. 3 The reduction in the total yield of US nuclear weapons over the years. Relative scale, 1972 = 1.00.

emitted following the impact of an asteroid surpasses the total energy and smoke released in a nuclear war by a factor of 1,000. In this scenario the massive amount of smoke may well have overcome the effects of atmospheric moisture.

By contrast, in a nuclear war, as was pointed out¹, the mass of smoke is 10^{-4} times the mass of H_2O in the atmosphere at the corresponding latitudes. Thus an "asteroid" winter seems plausible but hardly a nuclear winter.

Both Sagan and I are rightly worried and are rightly considering the damage a nuclear war may produce. We differ in many respects, but most particularly in my concern about the direct and the intended effects of nuclear war, including the local effects of prompt radiation, while Sagan has concentrated on side effects. These side effects are obviously dwarfed by an event for which there is geological evidence.

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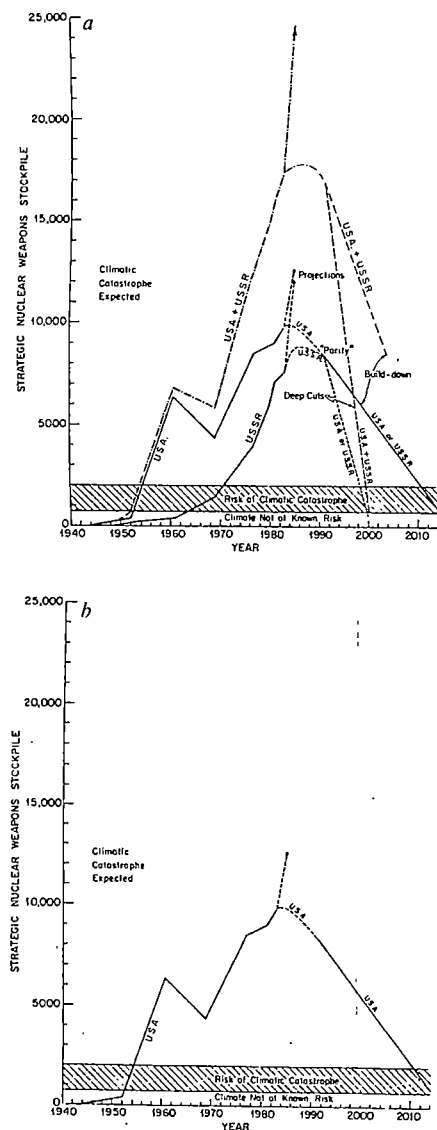


Fig. 1 a, Sagan's estimate of numbers of US and Soviet strategic nuclear weapons. b, An adaptation of Sagan's estimate of the numbers of US and Soviet strategic nuclear weapons.

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2. Turco, R.P., Toon, O.B., Ackerman, T.P. Pollack J.B. & Sagan, C. *Science* 222, 1283-1292 (1983).
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CORRESPONDENCE

Origin of AIDS

SIR—In their recent review article in *Nature* (3 October, p.395), Wong-Staal and Gallo proposed that the HTLV-I virus originated in Africa and was brought to other endemic areas by commercial and slave trading dating back to the sixteenth century.

If this is true, I fail to see how the HTLV-I virus could have reached Alaska and Northern Scandinavia where it has been detected, and southern Japan where it is endemic. Japan was virtually cut off economically and politically from the rest of the world until the latter part of the eighteenth century. There was hardly any commercial trade between Japan and Africa before the twentieth century.

It is also important to emphasize that the presence of the HTLV-I virus in the vast expanse of continental Asia cannot be ruled out due to lack of epidemiological studies in the area.

Recent studies by the same authors now show that retroviruses highly similar to the human acquired immune deficiency syndrome (AIDS) HTLV-III virus occur not only in African green monkeys and macaques but also in some flocks of European sheep¹. These animals can all show AIDS-like symptoms with advanced infection. Hence the possibility of humans acquiring AIDS worldwide from animal sources cannot be overlooked. Indeed, such a possible origin of human AIDS has recently been proposed by Lewin².

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Biotechnology aids

SIR—In your report (*Nature* 6 June, p.448) on plans for the computing facilities at the European Molecular Biology Laboratory (EMBL), only passing reference was made to the "Biosequences" workshop held at Grottaferrata (near Rome) on 24–25 May.

EMBL's plans were one contribution in a lively and wide-ranging debate on criteria and guidelines for the selection, by the European Economic Community (EEC), of projects contributing to the development of user services and research infrastructure for biotechnology in Europe. The workshop, sponsored by EEC, the Italian National Research Council and the University of Bari and organized by the latter, was timely in relation to EEC's current funding programmes for bio-informatics and for the development of the market in high quality information services. It also offered interesting comparisons and contrast between the various national activities of the

United States, the United Kingdom, France, Japan, Italy and Ireland.

Of interest was the emphasis by both structural researchers (A. Lesk of MRC, Cambridge) and molecular evolutionists (C. Saccone of Bari, R. Grantham of Lyons) on the need for a comprehensive approach, integrating all the diverse sequence, structural and biological data available in constructing and refining models and hypotheses (Compare with Lesk, A. "Coordination of sequence data", *Nature* 314, 318; 1985). The paper by B. Keil (Pasteur Institute) on the use of the proteolysis data bank to define the fixation sites of proteolytic enzymes illustrated the power of organized data.

These comprehensive and necessarily multidisciplinary approaches and the cost and difficulty of developing sophisticated and efficient software (for example C. Rawlings of ICRF on artificial intelligence approaches to structure prediction) and hardware (for example A. Coulson of Edinburgh on the application of distributed array processors to sequence comparison), lead to emphasis on better communication, networking and resource sharing between European centres. Training, users' clubs, electronic mail, software portability and on-line access were typical of the topics commended for support, complementing the centres and activities funded from national or EMBL resources. The workshop proceedings will shortly be available from the Research Directorate of the Commission of the European Communities (200 rue de la Loi, B-1049 Brussels).

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Creationism unloved

SIR—In his letter (*Nature* 316, 184; 1985) C.K. Pallaghy, a senior lecturer in the school of biological sciences, La Trobe University, attributes the paucity of overt support for creationism by bona fide academics in reputable scientific departments to hostility to creationists by the hierarchy of their institutions and by their colleagues, and to fear of loss of respect and prestige generally. This attitude to creationists is hardly surprising in view of the methods they and their organizations adopt in promoting their fundamentalist religious views under the guise of "creation science".

Not content that our taxes are being used, through school grants, to support their fundamentalist Christian schools, they lobby vigorously for "creation science"

to be taught in the state schools and have already scored some success in Queensland. The creationists' acute aversion to evolutionism is such that they claim the *Genesis* creation stories to be the literal truth, despite their marked resemblance to Babylonian myths and to the mixtures of guesswork, poetic fantasy and folklore compiled by other races to account for the origin of man. So it is that Pallaghy, replying to a critic, had a letter last year in the Melbourne Age containing the passage, "first, does he realize that Noah's ark was immense (450×75×45 feet)? Its tonnage would have supported 4,000 fully grown African elephant bulls. There would have been plenty of room for all had God put young animals on board."

Everyone will agree with the creationists' right to believe whatever they like, but not with their wish to impose such beliefs on the young and vulnerable, in contradiction to the findings of the past several hundred years of intensely painstaking research — a priceless cultural heritage.

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PhD theses

SIR—I wholeheartedly agree with Beverly Halstead (*Nature* 29 August, p.760) concerning the relatively trivial return of a British PhD thesis volume from the comparatively substantial cost (invested?) for the purpose of its production.

The advocated prerequisite for the PhD award of publication of scientific articles arising from the thesis material is, in fact, similar to the system operated by many universities and colleges in both Scandinavia and the United States. Indeed, some overseas establishments demand only that the material be published in a journal without requiring a formal bound volume. No doubt some would object to the adoption of such systems in this country but at least the various funding bodies would be able to assess the value of their investment more readily as well as providing additional incentives to the PhD candidate.

Incidentally, I was enlightened by Beverly Halstead's mention of costs for technical assistance during the period of postgraduate research. This clearly does not apply in every case since neither myself nor the postgraduate students in this and other departments were offered the benefit of technical assistance. We were simply informed that the PhD was supposed to be "an individual effort".

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Multiple sclerosis and viruses

Evidence that human T-cell lymphotropic viruses are associated with multiple sclerosis may lead to identification of viruses that cause the disease — or to an addition to the list of failed candidates.

It is not every day that the Faroe Islands are mentioned in the scientific literature, but no contemporary review on the causes of multiple sclerosis is complete without a mention of them. While the disease was unknown in the Faroes before 1940, a mini-epidemic was recorded in the years after British troops were garrisoned there in 1940. There seems little doubt that an infectious agent was involved; it is now conventional to suppose that multiple sclerosis can be initiated by viral infection in genetically susceptible individuals. But which virus or viruses are the culprits?

Frustration has marked the hunt. By some counts, twelve separate viruses have at one time or another been implicated as initiators of multiple sclerosis. But in no case has the case been made to stick. So the first evidence of the frequent exposure of multiple sclerosis patients to a new virus type is bound to excite an equal mixture of interest and scepticism. Publication of the data, moreover, is bound to quicken the pace of the further research that will show whether the new type of virus is genuinely an initiator of multiple sclerosis, or whether it will join its dozen or so predecessors on a list of suspects. It is in this spirit that we publish, on page 154, the tantalizing but inconclusive evidence from Hilary Koprowski, Robert Gallo and their colleagues of an association between multiple sclerosis and the human T-cell lymphotropic viruses (HTLVs).

This provisional group of viruses comprises three members. HTLV-I was the first to be discovered, in 1980, and is the cause of adult T-cell leukaemia discovered in Japan. HTLV-II is a variant of HTLV-I; and HTLV-III (also known as lymphadenopathy-associated virus, LAV) is the cause of acquired immune deficiency syndrome (AIDS). Although HTLV-III lives up to this name in some ways, notably a propensity to infect human T lymphocytes, its nucleic acid sequence is not related to that of HTLV-I or HTLV-II. Another view, now strengthening, is that HTLV-III is more related to the lentiviruses than to the other HTLVs. Evidence comes from both structural comparisons and the fact that the HTLV-III can be found in brain tissues of certain AIDS patients (Shaw, G.M. *et al. Science* **227**, 117; 1985). Lentiviruses are animal viruses that cause progressive demyelinating diseases of the brain and spinal cord. Indeed, the disease caused in sheep by visna virus, the most studied of the lentiviruses, is often

said to be the best naturally occurring model for multiple sclerosis.

Inevitably, therefore, attention has been turned to the possibility that HTLV-III or a related virus is the cause of multiple sclerosis. Ironically, the evidence of Koprowski *et al.* is if anything more suggestive of an association between multiple sclerosis and HTLV-I or HTLV-II than with HTLV-III. Indeed there is no uniform response among the patients studied to tests for any of the three HTLV types; the authors say this conceivably indicates the presence of one or more entirely new HTLV-like viruses, possibly including a virus that combines some of the features of all three known HTLVs.

Leaving aside the identity of the viruses responsible for the signals detected in the tests, what and how strong are the signals, and to what extent can they be interpreted as evidence that the viruses in any way cause the disease? The signals are of two distinct types: the presence in the blood and cerebrospinal fluid of antibodies that cross-react with HTLV proteins, and the presence in cells cultured from cerebrospinal fluid of HTLV RNA. The former indicates that the viruses have been present, the latter that they are still present and active.

There are three problems in interpreting the antibody data. First, although it is clearly shown that the concentration of antibodies that cross-react with some HTLV proteins is greater in most serum and cerebrospinal fluid samples from multiple sclerosis patients than in various controls, such antibodies are not invariably present. Second, no consistent picture emerges as to which HTLV the antibodies are most closely related, although HTLV-I is much more frequently and usually more strongly implicated than HTLV-III. Third, and most important, it is already known that elevated concentrations of antibodies to measles and Epstein-Barr virus are frequently found in the cerebrospinal fluid of multiple sclerosis patients, throwing doubt on the significance of raised antibodies to other viruses.

For the data that indicate the presence of RNA that is related to HTLV RNA in cultured cerebrospinal fluid cells, at least there is no consistency of HTLV type. In the 4 out of 8 cases where RNA has been detected, it is related, though not very closely, to RNA of HTLV-I but not HTLV-III. When present, the RNA is detected in less than one cell in ten thousand,

which may at first seem too low to be of significance, but this rate is similar to the frequency of HTLV-III RNA in T cells of AIDS patients. Moreover, it is a general observation in chronic virus infections, including visna and measles, that only 0.1 to 1.0 per cent of cells in any tissue contain viral genetic information. Much more of a problem is that the controls are so far limited to two healthy subjects, neither of whom had HTLV-related RNA in the cells of their cerebrospinal fluid.

When Ashley Haase and his colleagues first reported measles virus RNA in several multiple sclerosis brains (*Science* **212**, 672; 1981), the data, together with those on elevated antibodies to measles virus in multiple sclerosis, made the measles virus a strong candidate as the initiator of the disease. But further investigations revealed that measles virus RNA can be detected almost as frequently in the brains of patients with other neurological and non-neurological diseases as in those with multiple sclerosis, weakening the case for the involvement of measles virus in multiple sclerosis, though not excluding it.

Clearly, more data, and particularly more controls, will be needed before the case for an HTLV-type virus as the initiator of multiple sclerosis can be realistically assessed: the National Multiple Sclerosis Society in New York has already organized several laboratories to begin participating in studies of the putative association with HTLV. There will also be intense efforts to try and isolate the virus or viruses that are the source of the HTLV-I-like RNA. Until that can be achieved, there can be little progress in understanding the variability of the HTLV-related antibodies in multiple sclerosis.

Even if a virus can be proved to be implicated in multiple sclerosis, it is likely to be merely the initiator of a chain of immunological events leading to demyelination of nerve fibres rather than a direct cause. It seems probable that the initial viral infection somehow triggers an autoimmune reaction against components of the myelin sheath of nerves. "The baroque complexities of contemporary immunology in its application to multiple sclerosis", as Byron Waksman puts it in summarizing current research on page 104, will require considerable unravelling before it is clear how the disease progresses from the triggering event.

Peter Newmark

Astronomy

When is a star a superstar?

from C. Martin Gaskell

How massive and luminous can a star be? The traditional upper limit to the mass of a stable main-sequence star is about 60 times that of the Sun, but superstars with masses thousands of times greater than this have been seriously considered for the power sources in quasars (or at least as possible progenitors of the supermassive black holes currently favoured as these power sources). The object R136a in the Large Magellanic Cloud has been claimed to be such a superstar. The latest observations¹, however, show that it is not a single object but a small compact cluster of bright stars, some of which nonetheless have a luminosity that exceeds that of the brightest known star in our Galaxy.

The dominant feature of the Large Magellanic Cloud — an irregular companion galaxy to our own Milky Way — is a gigantic region of star formation, visible to the naked eye, and known as 30 Doradus (the name comes from the 1712 star catalogue of John Flamsteed, the first astronomer royal). This nebula — often called the Tarantula — is the biggest region of star formation in our local group of galaxies. It is over 3,000 light years across and contains half a million solar masses of gas. In its centre are some extremely bright stars, dominant among which is HD38268. More commonly known as R136 (it was number 136 in a list of stars published by the Radcliffe observatory² in 1960), it is putting out enough ultraviolet radiation to ionize almost the entire Tarantula nebula.

What is R136? Studies at the beginning of the century showed it to have the spectrum of a very hot O star. In 1973, N. R. Walborn suggested that it was not one star but a small compact cluster of very luminous stars³. He argued that compact systems of a handful of bright stars within a light year or so were often found in the very centres of giant star-forming regions. The four stars known to amateur astronomers as the Trapezium, in the Orion nebula, are the nearest example. Walborn argued that if a system like the Trapezium were transported to the distance of the Large Magellanic Cloud, it would resemble a single star on photographs. Further work showed that R136 does have several components⁴ but two groups suggested that the brightest component (R136a) was a single massive superstar with a mass of 2,000–4,000 solar masses^{4,5}. Such a star would be very interesting as it would far exceed the conventional upper limit to the masses of normal stars. This limit, first derived by A. S. Eddington⁶, and named after him, occurs because the intensity of radiation becomes so great that radiation pressure exceeds gravity. R136a is in fact

losing mass at an enormous rate: Cassinelli, Mathis and Savage estimate it loses the equivalent of the entire mass of the Sun in 3,000 years⁵.

Better spatial resolution was needed to see if R136a is indeed a single superstar. The resolution of photographs taken by telescopes from the Earth's surface is set by turbulence in the atmosphere, and the very best photographs of R136a, taken in Chile, have a resolution of 0.7 arcseconds⁷. It was C. E. Worley, of the US Naval Observatory, who realized that R136a had already been resolved by visual micrometer measurements made 60 years ago⁸ but overlooked by modern spectroscopists. Worley, himself, made further visual observations in 1983 and published the results in a one page paper¹⁰ (perhaps the last time anyone will publish a visual micrometer observation of a double star in the *Astrophysical Journal Letters*). Worley's observations confirmed that R136a is actually two stars separated by 0.49 arcseconds with one (R136a₁) being twice as bright as the other (R136a₂). R136a₁, if a single star, would still be a very impressive object of perhaps 750 solar masses⁷. But is it a single star?

The issue has finally been settled by G. Weigelt and G. Baier using the technique of holographic speckle interferometry¹.

From 8,000 short-exposure pictures of R136, taken with the Danish 1.5 m telescope at the European Southern Observatory, they were able to reconstruct the true image of R136a because of the presence of the point sources R136b and R136c in the same field (holographic reference stars). Their conclusion is that R136a is actually a double star with a 0.10 arcsecond separation, and that there are at least 8 stars within a circle 1 arcsecond in diameter. Weigelt and Baier have therefore shown that R136 is indeed a complex of stars, much like the Trapezium in Orion, as Walborn had suggested.

Although nobody now believes 30 Doradus contains a 2,000 solar mass superstar, R136a remains highly interesting. Its three brightest components are each probably of more than a hundred solar masses, brighter than any star in our Galaxy. Many questions about the birth, life and death of such stars remain. □

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Embryology

Interacting systems in amphibia

from Hugh Woodland and Elizabeth Jones

AMPHIBIAN embryos have long been a favourite experimental material for embryologists because their large size and independent development makes them particularly amenable to techniques like micro-injection and grafting. They are therefore particularly suitable for finding out how the variety of cells in the very early embryo differentiate. This kind of information is virtually non-existent for *Drosophila*, another developmental system much in favour at the moment. *Drosophila* work relies mainly on mutants and those available affect higher orders of pattern, rather than the basic events of cyto-differentiation. Thus, although the power of genetics cannot be applied to the amphibians, they still have a very important place in embryological research. Moreover, new life has very recently been injected into the study of amphibian embryology by the introduction of cloned DNA and antibody reagents which distinguish particular types of differentiation.

Until now, it has been necessary to rely

on purely morphological criteria for identifying tissues in amphibian embryos and, in many kinds of experiment, tissues may be very abnormal and their identification hard to justify, especially to those without extensive histological experience. Sometimes, as where cell division is blocked with agents that disrupt the cytoskeleton, the correct morphology of the tissue could not possibly appear. Cloned DNA molecules or antibodies (mostly prepared from *Xenopus* embryos) are being introduced to circumvent these problems. The DNA gives very accurate quantitative information but, since nobody has yet developed a viable method of *in situ* hybridization for early stages of amphibian development, spatial information is limited. The reverse is true of antibodies, which may readily be used for the immunohistological staining of sections.

How are the main tissues of the embryo generated? At the time of nervous system formation, all vertebrates have the structure shown in Fig. 1c. The considerable

tissue diversity is generally believed to originate from heterogeneity in the egg's cytoplasm, which is subsequently partitioned into different cells during cell division (cleavage). The small number of initial cell types thus formed are thought to interact with each other to generate further complexity. The original example of such an interaction, the 'induction' of the nervous system, was discovered by Spemann and Mangold over 50 years ago. Induction is the process in which the pathway of development of tissue A is changed by interaction with tissue B while that of B is unaffected. In the case of the nervous system, ectoderm is the responding tissue; in isolation it forms only epidermis, but placed with dorsal mesoderm (the future notochord and segmental voluntary muscle blocks) it forms nervous system (Fig. 1*g-i*). This invites the question of what causes the mesoderm to develop, and whence comes its dorsal-ventral polarity. This problem, which has received considerable attention lately, is especially interesting because the mesoderm is responsible for so much of the internal complexity of the body.

It was originally thought that the mesoderm arises from a special type of cytoplasm in the zygote, but there is no unequivocal evidence that the very early embryo contains more than two cell types, the ectoderm and the endoderm. The former develops from the pigmented half of the egg, the latter from the non-pigmented yolk half. In the 1960s, the field was revolutionized by Ōgi¹ in Japan and Nieuwkoop² in Holland. Nieuwkoop, who has not received the recognition appropriate to the importance of the work, concluded that the mesoderm is mainly or wholly formed by an induction in which the yolk endoderm induces its formation from ectoderm (Fig. 1*a*). This was shown by experiments in which animal and vegetal cells, neither of which form mesoderm in isolation, were removed from an embryo and grafted together (Fig. 1*d, e*). The result was the generation of mesodermal tissue (Fig. 1*f*), from which it can be concluded that a variety of mesodermal tissues may be induced from ectoderm that would normally form epidermis and nervous system. This basic result was soon supported by Nakamura's group in Japan³.

Very recently, cell-specific markers have provided a sophisticated confirmation of this process. John Gurdon, Tim Mohun and their colleagues in Cambridge have used cloned actin genes and an antibody to pick up the muscle component of mesoderm formed during mesodermal induction⁴; in Jonathan Slack's lab in Mill Hill, London, antibodies which react with muscle, epidermis and notochord have been used for the same purpose⁵.

These experiments have also more accurately established the dynamics of mesoderm induction. For example, even

in those experimental situations in which tissues have to re-establish communication, induction can take as little as 1.5–2.5 hours⁶. Furthermore, responsiveness of the ectoderm to induction and the ability of the endoderm to induce is lost in the early gastrula. It is important to know these kinds of details for at least three reasons. First, losses of responsiveness happen to be the first detectable signs that cells have adopted a particular path of development. Second, knowing when responsiveness and inductiveness occur should help in identifying the molecules responsible. Third, charting loss and gain of these properties will help in understanding the organ-building processes of early development. During the extensive morphogenetic movements of gastrulation, cells completely change their

that the agent is naturally relevant to mesoderm formation, even in the chick. The same is true of putative neural inducers isolated from amphibians⁷. In both cases, the next step is possibly to purify the putative inducers and generate monoclonal antibodies against them. These could then be tested for their ability to block induction and used in a strategy to clone the genes.

Oddly enough, the precise time when natural mesodermal induction occurs is still unknown. It might seem simple to take embryos at different stages, remove the cells normally expected to form mesoderm, and to find the earliest stage at which they develop, into, say, muscle. Unfortunately, throughout the likely period of induction, it is not possible to recognize any of the cells concerned. Fate mapping indicates that the general region

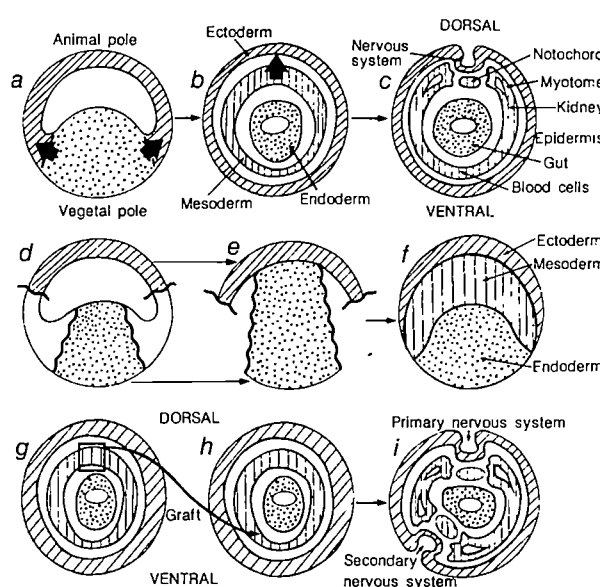


Fig. 1 Diagrammatic transverse sections of amphibian embryos in normal development (*a-c*) and in grafting experiments (*d-i*). Blastula stage embryo (*a*) with precursor ectoderm and endoderm, which interact (arrows) to form mesoderm between them. *b*, After gastrulation dorsal mesoderm induces (arrow) overlying ectoderm to form nervous system. *c*, Neurula stage, the main body organs are mapped. If the animal cells and vegetal cells are removed (*d*) and combined (*e*), they interact to generate mesodermal tissues (*f*). If dorsal mesoderm is removed from one embryo (*g*) and placed ventrally in another (*h*), the latter develops two sets of axial structures (*i*).

neighbours. If cells maintained a fixed response to a certain set of stimuli, or if the stimuli were always present, abnormalities could result. New phases of mesodermal induction can, and almost certainly do, occur later, for example during formation of the tail⁶ and perhaps the muscular cells of the iris.

We need to know if there is any unity in these processes, but there is unlikely to be much advance on this front until the molecular basis of at least one type of mesoderm induction is established. Progress has been slow in this direction. Although usable assays exist, they indicate that embryos at the appropriate stage contain only minute amounts of the substances that cause mesodermal induction. However, agents that cause induction have been isolated from more abundant sources, such as late-stage chick embryos or guinea pig bone marrow. A few micrograms of the chick agent have recently been purified and 10 nanograms of this 13,000 M_r protein have been shown to be sufficient to induce a nervous system⁷, though it remains to be proved

which forms mesoderm also contains cells which normally form endoderm or ectoderm, and which are capable of interacting to generate mesoderm up till at least the gastrula stage. Thus it is not possible to prepare an appropriate tissue fragment that is demonstrably incapable of undergoing new induction, even if the normal interaction has already occurred.

This same problem bedevils attempts to show that part of the mesoderm might be produced by an area of egg cytoplasm containing localized mesodermal 'determinants'⁸. Although the overall region that forms mesoderm in isolation contains probable ectoderm and endoderm precursors¹⁰, improved cell-marking techniques show that most of the mesoderm comes from the pigmented half of the embryo¹¹, which forms only epidermis in isolation. This strongly supports induction as the natural mechanism of mesoderm formation, since the pigmented region must develop into mesoderm only through the proximity of the yolk half of the embryo.

The mesoderm itself is obviously non-

homogeneous; its dorsal side contains the notochord and somites, as well as the neural-inducing ability, while the blood islands are ventrally located (Fig. 1b, c). Nieuwkoop, himself, first clearly showed that this vital dorsal-ventral polarity is imposed by the endoderm², experiments which have been elegantly refined by Bob Gimlich and John Gerhart in Berkeley¹². In its turn, the dorso-ventral asymmetry in the endoderm arises from cytoplasmic movements in the egg, polarized by the entry of the sperm — as discovered in the 1930s but now explored in some depth by the Berkeley group¹². The result is that the dorsal endoderm is formed on the side opposite sperm entry.

The simplest explanation of subsequent events is that there are three kinds of cell interaction that bring about mesoderm induction. First, (though not necessarily temporally so) there is mesoderm induction, which alone produces solely ventral mesoderm. The ventral nature of this effect is shown by obliterating the polarizing effect of the sperm on an embryo, for example by UV irradiation, whereupon a radially symmetrical embryo develops; most of the mesoderm forms blood cells, and none of it forms notochord or somites¹³. Second, the dorsal endoderm somehow instructs the overlying future mesoderm itself, to become dorsal, that is notochord and somite. This can be shown by transplanting pairs of blastomeres at the 32-cell stage¹², or by a modification of the operation shown in Fig. 1d-f, in which endoderm from future dorsal and ventral sides was transplanted separately^{14,15}. The third kind of interaction occurs within the mesoderm itself. Dorsal mesoderm influences the rest, perhaps through generating a morphogen gradient, to produce the dorso-ventral pattern of structures outlined in Fig. 1c. This is

demonstrated by the famous Spemann and Mangold experiment in which the dorsal mesoderm is grafted into a ventral position (Fig. 1g, h); a second set of notochord, somites and nervous system is generated, and the result is a 'siamese-twin' embryo (Fig. 1i). The influence of the graft on its surroundings is shown by the fact that the secondary set contains host cells as well as those of the graft¹⁵.

Sorting out the detailed cell and molecular biology of these interactions, as well as the generation of the initial cell heterogeneity by cytoplasmic determinants, needs simple experiments (like the grafts shown in Fig. 1d-f) that can be adapted to provide routine assays for the molecules involved. Fortunately, the development of such assays will be greatly facilitated by the cell-specific markers currently being developed, not least because they will make it possible to bypass time consuming histology. □

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Human disease

Mechanisms in multiple sclerosis

from Byron Waksman

THE splendor of the great stairs and ceilings of the Würzburg Residenz, created by Balthazar Neumann and Tiepolo, provided a fitting backdrop for a recent discussion* of the baroque complexities of contemporary immunology in its application to multiple sclerosis (MS), a crippling disease of young adults.

Susceptibility to MS seems to be under multigenic control, with links to *HLA-D* locus genes *DW2 (DR2)* on chromosome 6 (C. Jersild) and possibly to IgG allotype genes (*Gm-1, 17;21*) on chromosome 14 (J. Pandey *et al.*). A role for genes governing the T-cell receptor subunits and/or vasoactive amine sensitivity, suggested by

the results of animal studies (see below), remains conjectural, as is the significance of increased frequency of spontaneous and induced chromosomal translocation in MS cells (G.R. Sutherland).

The evidence that MS in susceptible individuals is initiated by viral infection, at least in some cases, is increasingly persuasive. For the epidemic in the Faeroe Islands that followed the arrival of British troops in 1940, it seems that approximately 2 years of exposure to the putative infectious agent was necessary and that the period from infection to onset of clinical disease was about 6 years (J. Kurtzke). Susceptibility was low before puberty. In informal discussion, it was mentioned that MS elsewhere is associated with infections

(measles, mumps, rubella, Epstein-Barr virus) caught significantly later in age than in HLA-matched controls (A. Compston, O. Andersen). In a well-studied cohort of patients, over 25 per cent of exacerbations were associated with such viral infections; from two weeks before to five weeks after infection, the exacerbation rate was increased more than threefold (W. Sibley).

Viruses such as measles, rubella, and varicella have been shown to sensitize T lymphocytes to myelin basic protein (MBP) in some subjects; this sensitization is very frequently associated with 'post-infectious' encephalomyelitis (R.T. Johnson). In a study of such encephalomyelitis associated with rubella, multiple T-cell clones obtained from cerebrospinal fluid showed equal reactivity with the virus and with MBP (P. Marquardt). Three independent computer searches have indicated the possible chemical basis for this cross-sensitization by demonstrating the presence of homologous peptide sequences in encephalitogenic regions of MBP and in antigens of measles, influenza, canine distemper, Epstein-Barr virus and several papova and adenoviruses (R. Fujinami, U. Jahnke *et al.*, G. Stoner).

A key point of current research is to determine the exact location where specifically-immune T Lymphocytes, circulating in the blood, first recognize myelin (or other) antigen, 'presented' in conjunction with major histocompatibility antigen (Ia). In agreement with the findings in animal studies, Ia has now been demonstrated on the endothelium of blood vessels within MS lesions and on activated astrocytes near the edges of lesions, as well as on the population of infiltrating macrophages (U. Traugott). MBP has also been demonstrated at these locations. Thus presentation sufficient to trigger T cells in the blood, or such T cells as enter the perivascular space, may occur at the luminal surface of the endothelium or the foot processes of the astrocytes. In older lesions, presentation may take place on the cell bodies of astrocytes, or other glia, and on the surface of macrophages.

The early MS lesions, triggered in the white matter of the central nervous system by these reacting T cells, show vascular injury with leakage of fluid, invasion of the tissue by a mixed population of lymphocytes and macrophages, and myelin destruction (C. Raine, H.L. Weiner). T lymphocytes, mostly of the T8⁺ class but with some T4⁺, predominate. Haematogenous macrophages play the principal role in myelin destruction at the advancing edge of the lesion (J.W. Prineas) by a mechanism that seems to involve receptor-mediated endocytosis. That the ligand for the receptor may be antibody is suggested by immunoglobulin 'capping' on many of the actively phagocytic macrophages. But extracellular lysis of myelin is also seen: oligodendrocytes at the edge of the lesion look healthy, while those in contact with lymphocytes or macrophages show mor-

*"Multiple Sclerosis — Current Experimental Work and Some Clinical Perspectives" Würzburg, 29–31 August, 1985.

phological evidence of cell damage.

T cells found in the cerebrospinal fluid may reflect events in the central nervous system. Both T8⁺ and T4⁺ types are present and many carry markers characteristic of long-term cell lines (Weiner). There is one report of cells specific for MBP in very acute cases of MS (R. Lisak and B. Zweiman), but most studies of specificity suggest that the T cells are polyclonal and that many are MHC-autoreactive (A. Salmi, G. Birnbaum). The cerebrospinal fluid also contains raised levels of γ -interferon, even during clinical remission (R. Hirsch). The B cells and plasma cells of the lesions and the cerebrospinal fluid show a polyclonality that is comparable to that of the T cells and affects all the major immunoglobulin classes (K.P. Johnson, H. Link). The much debated 'disappearance' of T8⁺ cells from the peripheral blood early in acute attacks (J. Antel and B.G.W. Arnason) has been attributed either to their actual migration into the target tissue (Weiner) or to modulation of their phenotypic markers by, for example, prostaglandins released from activated monocytes in the circulation (P. Dore-Duffy, J. Merrill). A striking new finding is the absence, in most patients, of cytotoxic T-cell precursors specific for measles virus (H. McFarland). There is no comparable deficiency for influenza virus.

For many of these crucial new findings, the study of animal models with conditions that resemble MS has led the way. Experimental allergic, or autoimmune, encephalomyelitis (EAE), induced by immunization with MBP or proteolipid protein, may take a chronic relapsing or progressive form, particularly if immunization is at the time of weaning (equivalent to puberty) (Raine, D. McFarlin, F. Lublin, T. Tabira). Certain temperature-sensitive mutants of the JHM virus produce a subacute or chronic inflammatory demyelinating disease in rats that is associated with sensitization against MBP; lymphocytes from these animals produce typical EAE in normal recipients (H. Wege, R. Watanabe). Theiler's murine encephalitis virus (TMEV) produces similar disease in SJL mice and certain other strains (H. Lipton); although there is an immune response against myelin, the continuing reaction may be directed at persistent viral antigen (Lipton, P.L. Lampert).

The use of recombinant inbred mouse strains has shown there to be multigenic control of susceptibility to the MS-like disease, as well as to its intensity and character, in both EAE and TMEV models. For EAE, both high-responder and low-responder strains have been found (Lublin). The high responders, most frequently associated with *H-2^s*, readily develop chronic relapsing or progressive disease. Earlier studies in acute EAE had mapped the responsible *H-2* gene to *I-A* (R. Fritz). Genes controlling vascular sensitivity to vasoactive amines are also important (S. Linthicum). For the TMEV sys-

Acute phase

Systemic virus infection (measles, varicella, influenza)

Primary (cross-reactive) immunization to MBP

Circulating T lymphocytes specific for MBP

Systemic interferon (α , β , γ) response

Activation of vascular endothelium (and astrocytic foot process?) by viral particles and/or by γ -interferon

Expression of Ia in vascular endothelium body-wide and in CNS

Acute phase events in CNS vessels

Expression of Ia and presentation of MBP

Dual recognition by MBP-specific T cells

Activation of T cells leading to nonspecific secondary inflammation

Leakage of fluid producing oedema

Invasion of tissue by monocytes (macrophages), T cells and B cells

CNS tissue damage by oedema and invading cells

Demyelination by activated macrophages

Damage enhanced by local antibody forma-

tion, complement from circulation, free oxygen radicals (?)

Arrest of conduction, due to fluid pressure, loss of myelin, and perhaps T-cell lymphokines and free MBP

Chronic phase

Activated T cells produce IL-2 and γ -interferon locally

Activation of astrocytes by γ -interferon

Expression of Ia, release of IL-1 and prostaglandins by astrocytes invading macrophages

Proliferation, synthesis of GFAP, gliosis

Activation of Ia-reactive T cells in response to Ia, IL-1 and IL-2 in lesions

Persistent foci of Ia-reactive T cells

Continuing nonspecific secondary inflammation

Systemic virus infection-reactivation of process

Astrocyte activation by systemic γ -interferon

Renewed activity of local Ia-reactive cells

Enlargement of existing lesions

New lesion formation, as in acute phase

MBP, myelin basic protein or other myelin antigens; Ia, major histocompatibility complex antigen (Class II); IL-1 and IL-2, interleukins-1 and 2; GFAP, glial fibrillary acidic protein; CNS, central nervous system.

tem, an important genetic control, related to the intensity of the T-cell mediated response against viral antigen, maps to *H-2D*; another seems to be related to the gene encoding the β -chain of the T-cell receptor.

Lines or clones of T cells specific for MBP produce acute or chronic EAE when activated (with mitogen or specific antigen) *in vitro* and transferred to normal syngeneic recipients (I.R. Cohen, H. Wekerle). The delay of 72–96 hours before the first inflammatory cells appear in the central nervous system (R. Meyermann) can be accounted for by the time required for the transferred cells to produce heparanase, which permits them to penetrate the vessel wall and enter the tissue (Cohen), and to produce γ -interferon, which activates vascular endothelial cells and astrocytes. The delay includes time for both cell types to synthesize and express Ia, which can be demonstrated on macrophages infiltrating lesions (W. Fierz, Sobel).

An important new finding is that purified endothelium from brain vessels can be stimulated *in vitro* by activated T-cell supernatants or purified γ -interferon to express Ia and present antigens such as MBP to specifically sensitized T-cells (McFarlin). Similar data for purified astrocytes have been reported (B. Fontana). The importance of presenting myelin antigen in association with Ia is clearly established in experiments with cell lines transferred from, or to, either of two rat strains and their F1 hybrid (C. Lington). JHM virus particles, however, are capable of directly activating astrocytes without mediation by γ -interferon (P. Massa). Manoeuvres which stimulate systemic γ -interferon and/or reduce suppressor T-cell activity trigger relapse, even in normally resistant animals, such as Lewis

rats that have recovered from acute EAE. An important confirmatory finding is that MBP-specific suppressor cells are present in the spleens of sensitized rats during periods of remission but not during exacerbation (W. Lyman and C. Brosnan).

A hypothetical sequence of events in MS, which merges findings reported at the symposium with reports in the recent literature, is presented in the table. Several points of attack for potential therapies are suggested by the scheme. It would be worth testing the value of monoclonal antibodies directed at phenotypic markers or characteristic activation antigens of appropriate T-cell subsets; at the T-cell receptor or its subunits, particularly idiotypes related to specific myelin antigens; or at Ia (Weiner). Hybrid or toxin-linked antibodies may be advantageous. Other leads come from studies of EAE. Vaccination with MBP-specific lymphocytes, inactivated with X rays or mitomycin C, induces resistance (idiotypic tolerance?) to actively induced EAE (Wekerle); lymphocytes inactivated by hydrostatic pressure (which changes the physical state of membrane glycoprotein) are particularly effective, inducing a resistance that is transferable with T cells (Cohen); prolonged treatment with MBP plus galactocerebroside not only turns off chronic EAE but promotes remyelination, perhaps due to oligodendrocyte stimulation by antibody to galactocerebroside (Rodriguez).

All in all, participants at the meeting were imbued with a sense of optimism that both the complex scientific problems related to MS and the practical issues of its prevention might be nearing solutions. □

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Cell metabolism

Organization in the cell soup

from Hans V. Westerhoff

METABOLITES moving directly from one enzyme to the next, enzymes sticking to each other and to membranes, intracellular water diffusing freely, energetics and kinetics not matching actual metabolism: in a recent workshop on *The Organization of Cell Metabolism*^{*}, these and related observations fell into a coherent picture. Rather than a thick, homogeneous soup consisting of metabolites and proteins at high concentration, the cell sap emerged as a bouillon with vermicelli. The vermicelli corresponds to a lattice of structural proteins to which cellular enzymes are adsorbed; metabolites move directly between the enzymes adsorbed next to each other, and where available, membranes can take over the role of the lattice.

As examples of the organization of metabolism through the dynamics of enzyme kinetics itself, glycolytic oscillations in yeast (B. Hess, Max Planck Institute, Dortmund) and oscillations in cyclic AMP concentration and movement in *Dictyostelium discoideum* (A. Goldbeter, Université Libre Bruxelles) were discussed. The models assumed the enzymes to be dissolved in a homogeneous solution of metabolites. However, the highlight of the workshop was the understanding of the spatio-temporal organization of metabolism arising from the breakdown of this very assumption. For instance, the *in vitro* desorption of NADH from α -glycerolphosphate dehydrogenase was reported to be greatly accelerated by excess lactate dehydrogenase, suggesting direct transfer to that enzyme. In general, direct transfer of NADH seems to occur between enzymes that bind it in the *syn* conformation and those that bind it in the *anti* conformation, which makes sense mechanistically (S. Bernhard, University of Oregon). In some cell types, most of the hexokinase is bound to a pore-forming structure in the mitochondrial outer membrane; glycerokinase added to a mitochondrial preparation does not effectively compete with the bound hexokinase for mitochondrial ATP (J. Wilson, Michigan State University).

These are extreme cases of 'metabolic channelling' — the transfer of metabolites from an enzyme molecule producing them to an enzyme molecule consuming them without equilibration with a 'pool' of the same metabolite. Such channelling can be either 'static' (where the two enzymes form a steady complex), or 'dynamic' (where metabolite transfer is contingent on the enzymes colliding).

In vitro, association of enzymes is often rather weak and dependent on low ionic strength (L. Kanarek, Vrije Universiteit

Brussel). But if the enzymes also bind to a support, the ensuing cooperative binding may suffice for physiological conditions. The inhibitory effect of oxaloacetate on the binding of citrate synthase to submitochondrial particles, suggests that association of enzymes may well be a point of metabolic control, which would explain why such association tends to be weak (P.A. Srere, Veterans Administration Medical Center, Dallas).

In view of this lability of enzyme supercomplexes, assay methods for channelling in intact systems have to be either delicate, such as the 'gentle' opening of cells that is necessary if cellular enzymes are subsequently to be shown to co-elute on chromatography columns (P.D.J. Weitzman, University of Bath), or based on functional properties implied by channelling. Several examples of the latter were presented. First, there is more rapid increase in specific activity of intracellular proteins than in that of intracellular amino acids upon extracellular addition of labelled amino acids (D.N. Wheatley, University of Aberdeen). Second, lactate is produced in toluene-permeabilized cells from glucose-6-phosphate but not any other added glycolytic intermediate (D.B. Kell, University College of Wales). Third, intermediary metabolites injected into frog oocytes fail to lead to the isotope dilution expected if metabolism were not channelled (T. Ureta, Universidad de Chile). Spatially inhomogeneous NAD(P)H fluorescence, after similar injections into mammalian cells, has been observed (E. Kohen, University of Miami). Another approach focused on the one-to-one relationship expected in static

channelling between enzymes producing a metabolite and enzymes consuming it, and on the implication that an inhibitor of the former enzymes would not affect the titre (with respect to the pathway flux) of an inhibitor of the latter. In membrane-linked free-energy transduction, this type of experiment provides an indication of channelling (D.B. Kell).

Nuclear magnetic resonance experiments have suggested that the inside of a cell resembles a thick but homogeneous soup. It is not obvious why channelling should occur in such a soup. Now, however, an alternative interpretation seems to prevail. The apparent seven-fold reduction in the self-diffusion coefficient of water is now thought to reflect the presence of barriers to (otherwise free) diffusion. Thus, shrinking of cells causes a reduction of translational, but not rotational, motion of a water-soluble electron-spin resonance probe (A.M. Mastro, Pennsylvania State University) and quasi-elastic neutron scattering suggests that, when measured over a few Angstroms, the diffusion coefficient of intracellular water is reduced only by a factor of three rather than seven (J. S. Clegg, University of Miami; C. Hazlewood, Baylor College of Medicine, Houston).

Against the background of structural information on the cytomatrix, there emerges a picture of an ultrafine structural lattice ramifying throughout the cytoplasm. Most of the intracellular protein is loosely attached to the lattice and water is free to move the short distances in between its threads. As was shown for artificially co-immobilized enzymes (A. Friboulet, Université de Compiègne), the proximity of the adsorbed enzymes will result in channelling. □

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Plate tectonics

Palaeozoic longitude problem

from A. G. Smith

GREAT strides have been made in quantifying global geology for Mesozoic and Cenozoic time — the past 250 million years. This success has depended on magnetic surveys of the ocean floor and on measurements of the magnetic field preserved in rocks on the continents. But the situation is quite different for Palaeozoic time (245–570 Myr), because virtually all the Palaeozoic ocean floor has gone down subduction zones, to be recycled into the mantle, thus destroying half of the critical data needed to reposition one continent relative to another. But a recent paper by L.P. Zonenshain, M.I. Kuzmin and M.V. Kononov (*Earth planet. Sci. Lett.*

74, 103; 1985), of the P.P. Shirsov Institute of Oceanology in Moscow, presents some new Palaeozoic reconstructions which are considered by the authors to have brought a solution within sight.

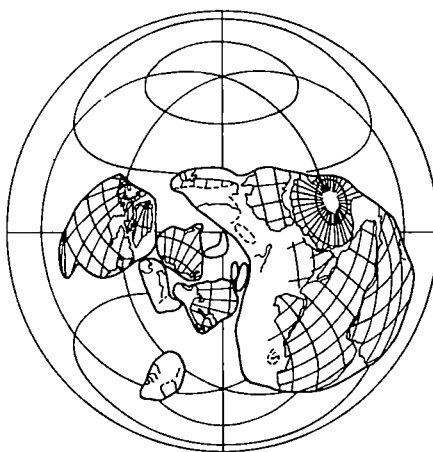
By appealing to plate tectonic theory and using Euler's theorem — which states that the motion of a tectonic plate can be represented by rotation about an axis through the Earth's centre and a point on the surface — geologists can make precise reconstructions of the relative positions of the Mesozoic and Cenozoic continents from the oceanfloor data. By assuming that the Earth's magnetic field has always been a dipole whose axis lies parallel to

the axis of rotation, they can put these reconstructions into their former latitudes from the inclination and declination of the magnetic field preserved in continental rocks of the appropriate age. There is no difficulty at all in estimating the relative longitude separation of the major continents for the past 180 Myr or so, as the longitude inherent in palaeomagnetic measurements is resolved by the ocean-floor data.

When two continents are fixed with respect to each other, their relative positions can be found by superimposing their apparent polar wander (APW) paths, which in theory should be identical. Gondwanaland has been reconstructed by this method, but then it was independently known that Gondwanaland was a single continent during the time interval for which the APWs were compared. If the motion between the continents is small, then applying the method will give a reconstruction that will contain relatively small errors. The problem in using this method for an arbitrary period in the past is knowing how much of the difference between two similar APWs is due to errors of measurement and how much to relative continental motion.

By adopting a criterion of minimum motion between the continents in time, Mesozoic and Palaeozoic reconstructions have been made on the basis of palaeomagnetism alone, and are not dissimilar to those based on oceanfloor spreading (Irving, E. *Nature* **270**, 304; 1977). More recently, R.A. Gordon, A. Cox and S.O'Hare (*Tectonics* **3**, 499; 1984) argued that Euler poles can be inferred from long APWs of the continents, though this use of the palaeomagnetic data contains hidden assumptions that should be more closely examined.

An alternative method of making reconstructions is to use the hotspot reference frame as a means of repositioning the continents. Provided hotspot traces are available for all the continents, they can be placed in the same frame independently of any other data and the longitude problem can be solved. R.A. Livermore, F.J. Vine and I (*Geophys. J. R. astr. Soc.* **79**, 939; 1984) have tested this idea as applied to the past 90 Myr and have shown that the differences in latitude inferred from the palaeomagnetic reference frame and the hotspot reference frame are less than 7°, with the pole enclosed by, or within a degree or two of, the α -95 circles of confidence for that interval. For the previous 90 Myr the difference progressively increases to nearly 20°, possibly because the hotspot frame for the Jurassic and Cretaceous periods is not well known. In cases of incomplete data, hotspots could be used to orient one or more continents, the remainder being oriented using palaeomagnetic Euler poles. If there are more than enough data, these methods can be combined appropriately to find the best solution. This is the approach the



A reconstruction of the positions of the continents in Cambrian time (roughly 550 Myr ago). Modified from Zonenshain, L.P. *et al.* (*Earth planet. Sci. Lett.* **74**, 103; 1985).

Russian group have attempted to use.

The problem with using the hotspot frame for Palaeozoic reconstructions is that no Palaeozoic hotspot traces had previously been suggested. What Zonenshain and his co-workers now propose is that three examples of igneous activity within Eurasia represent continental hotspot traces for the time periods 400–365 Myr, 400–320 Myr and 280–130 Myr. Taken together with approximate Palaeozoic reconstructions from palaeomagnetic Euler poles, these traces would enable the relative motions of the Palaeozoic continents to be found in the hotspot frame. The most striking difference between previous reconstructions and theirs is in the Cambrian. The continents are not only much closer together but are also in a different order, with Siberia lying between western

Europe and North America (see the figure).

One of the major problems in this kind of work is the scarcity of reliable and well-distributed palaeomagnetic data. Granted that, one question is what is the significance of the palaeomagnetic Euler poles? In essence, the method assumes that any long APW consists of arcuate segments joined together by cusps (Gordon *et al. op.cit.*). The cusps are interpreted as representing important periods of plate reorganization, the arcuate segments as periods of steady motion. The existence of cusps, or at least of sharp changes in the trend of an APW, is well known and almost certainly reflects a discontinuity in the motion of the continent concerned, such as its separation from, or collision with, another continent. But what of the arcs between? Obviously, any arcuate path on a sphere can be described geometrically by a pole and an angle. What is the evidence that such poles refer sufficiently closely to the same reference frame, so that they are useful guides for reconstructions of past continents as opposed to reference frames that differ significantly from continent to continent?

A second obvious question is whether there are any hotspot traces on other Palaeozoic continents. If not, does the systematic age progression in the igneous activity in Eurasia have a different origin, such as one related to progressive rifting? Whatever the eventual answers to these questions, the paper by Zonenshain *et al.* will stimulate much further research and discussion. □

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Palaeoanthropology

The earliest *Sivapithecus*?

from Eric Delson

THE search for a fossil ancestor (or even an extinct close relative) of the human lineage has met with mixed success over the past few decades. During the 1960s, the view that *Ramapithecus* was an ancestor of humans as contrasted with the great apes became dogma in anthropology¹, questioned by few workers and those usually on invalid grounds. Sharper attacks on this position characterized the 1970s, as details of molar enamel thickness, dental proportions and wear patterns revealed little difference between this taxon and *Sivapithecus*, its contemporary in 9–13 million year old rocks in India and Pakistan^{2,3}. Finds of more complete specimens attributable to *Sivapithecus* confirmed the similarity of these taxa and moreover permitted the inference that this expanded *Sivapithecus* group shared derived facial and dental features with *Pongo*, the living orangutan^{4,5}. This

left the fossil ancestry of human and African apes, joined phylogenetically by numerous generic and biomolecular studies⁶, open to question. Minority alternative views have arisen which suggest that *Sivapithecus* is ancestral (or at least closely related) either to all great apes and humans⁷ or to orangutans and humans alone⁸.

New evidence on the morphology and temporo-spatial distribution of *Sivapithecus* is therefore of great interest in this discussion. On page 173 of this issue, Leakey and Walker⁹ report and briefly describe new fossils from Buluk, Kenya, which they assign to an indeterminate species of *Sivapithecus*. This information is potentially exciting because the fossils are found in sediments that are more than 17 million years (Myr) old, as documented on page 175 by McDougall and Watkins¹⁰, far older than the earliest Eurasian fossils assigned to *Sivapithecus*. Based on this

age, Leakey and Walker now accept that *Sivapithecus* could well be the common ancestor of all later large apes, but realize that it tells little about the divergence of either the African lineage (Homininae: chimp, gorilla, human) or Asian (Ponginae: orangutan) lineage implied by molecular studies. But how accurate is their "unequivocal" identification and subsequent phyletic assessment, and what more (or less) can be inferred from these fossils?

Before these questions can be approached, it is useful to review the Buluk primates and other known occurrences of Miocene large apes. The fossil primate material from Buluk is rather scrappy, but four species are said to be present: three 'apes' of varying size and a more common species of Old World monkey. Although not cited by Leakey and Walker, the monkey has recently been described in detail by Meave Leakey¹¹, who assigned it to *Prohylobates*, a genus otherwise known only from slightly younger deposits in Egypt and Libya¹², rather than to *Victoriapithecus* of the Kenyan? Early to Middle Miocene (or about 17–15 Myr)^{13,14}. This apparent affinity with northern rather than eastern African taxa is mirrored in the remainder of the Buluk faunal list, which suggests either that the environment was more open than other Early Miocene sites nearby (perhaps akin to that of the Kenyan Middle Miocene sites Maboko and Fort Ternan) or that there was an influx of immigrant species.

Of the Buluk hominoid primates, a few specimens are referred to a small species, most to the new *Sivapithecus*, and an isolated tooth and dentulous mandible fragment to *Kenyapithecus wickeri*. The last taxon, intermediate in size, is best known from Fort Ternan but has also been reported from Maboko and nearby Majiwa¹⁵ and from Emuruiem (Nachola)¹⁶. There is still some controversy over the name and number of species of *Kenyapithecus* to be recognized but, despite previous synonymization with *Ramapithecus*, most authors now accept that *Kenyapithecus* is a distinct African mid-Miocene genus. While Pickford¹⁷ has grouped *Kenyapithecus* with the *Sivapithecus*–*Pongo* clade, Andrews¹⁷ places it on a separate lineage predating the Ponginae–Homininae divergence, because of its lack of derived *Pongo*-like features.

A number of other fossil 'populations' may also be relevant to understanding the Buluk specimens. In Asia, *Sivapithecus* is well documented in the Indo-Pakistan Siwaliks at about 12.5 Myr from remains with the distinctive facial characteristics seen in more complete younger specimens but, on dental evidence¹⁸, may extend back to over 14 Myr; recently, remains as young as 5.5 Myr have been assigned to this genus¹⁹. In Pakistan, the most complete fossils date to about 8 Myr. A large sample (probably representing a single species with high sexual dimorphism, contra Wu and Oxnard²⁰), of similar age from

China has also been placed in *Sivapithecus* and/or *Ramapithecus*²¹ but may require reassessment. The wide interorbital spacing and distinctive high-crowned incisors are not comparable to Siwalik *Sivapithecus*, although dental morphology is similar.

Sivapithecus has also been identified in Turkey and eastern Europe as old as 15 Myr, mainly on the basis of thick molar enamel, which Martin has shown to be a more complex character than previously thought²². The identification of *Sivapithecus* in Greece (see ref. 23) has been more widely accepted²⁴. De Bonis and Melentis²⁵ have now reported that the maxillary morphology of the Greek fossils, which they term *Ouranopithecus*, is more similar to that of hominines than *Sivapithecus* (see ref. 5). Kelley and Pilbeam²⁶ have suggested that the morphology thus revealed was derived and they have linked *Ouranopithecus* to hominine ancestry, but I prefer the de Bonis and Melentis interpretation that hominines share the ancestral morphology of this region, with the pongines like *Sivapithecus* being derived. Given that *Ouranopithecus* does share other derived features with *Sivapithecus*²⁴, it seems best to retain all the Eurasian thick-enamelled Miocene apes in a single clade.

In the light of this diverse assemblage of African and Eurasian Miocene larger hominoids, it is somewhat surprising that Leakey and Walker⁹ only compare the Buluk fossils to *Proconsul* and the Siwalik *Sivapithecus*. The novel faunal elements would suggest a wider search for comparative samples. Based upon the description and a brief examination of casts (courtesy of Walker), it seems to me that at least one alternative interpretation of the Buluk material is possible, if not more likely — that it represents a large *Kenyapithecus*. Pickford¹⁷ has presented new metrical data on *Kenyapithecus* which support the idea that the Buluk *Sivapithecus* is the male of a species whose female is identified there as *Kenyapithecus*. For example, his estimate of the length of a male upper canine of *Kenyapithecus* is slightly greater than that given for the Buluk male, while molar lengths are comparable to, or slightly smaller than, those estimated for Buluk from roots; Leakey and Walker⁹ do not give the direct measurements of the few relatively complete molars they have.

More to the point is the comparison of maxillofacial and mandibular morphology and enamel ultrastructure. The Buluk mandible is stated to be "remarkably similar" to an edentulous one from the Pakistani Siwaliks, but while this is so by comparison with *Proconsul*, there is little to differentiate among the Eurasian forms, all of which share superior and inferior transverse mandibular tori as well as a deep and thick corpus. The Buluk maxilla is not specifically compared with any Siwalik specimen, but the described morphology does read like that seen in several Asian *Sivapithecus* faces. It is not clear,

however, that the described pattern is unique to *Sivapithecus* or is as well developed in Buluk as in the Eurasian fossils. An enamel thickness of 1.5 mm is given for the cuspal region of one broken, but little worn, upper molar, somewhat less than the 2–2.5 mm recorded for Pakistani *Sivapithecus*²⁸. Martin²² has shown that ultrastructural detail (unknown in this case) as well as size normalization, is needed to make an accurate estimate, of the enamel pattern, but the Buluk fossils may present the "intermediate-thin" pattern, predicted by Martin but not yet observed. (Neither *Kenyapithecus* nor *Dryopithecus* has yet been analysed.)

The essence of Leakey and Walker's comparative analysis is that since the Buluk fossils are not *Proconsul*, they must be *Sivapithecus*. While the first part of this syllogism is unquestioned, their conclusion is by no means inescapable. The lack of even superficial comparisons with *Kenyapithecus*, with the Moroto maxilla (which has long been assigned to a large species of *Proconsul*²⁷, but may be of Middle Miocene age⁵ and thus rather younger than most of that taxon) or with *Dryopithecus* is surprising. It would seem, in fact, that an allocation to *Kenyapithecus* would be both more biologically parsimonious and reasonably supported by the morphological evidence. That *Kenyapithecus* is positioned close to the divergence of Ponginae and Homininae is thus reaffirmed, but the implications for the broader phylogeny of large hominoids in terms of palaeontology or molecular-clock calibrations are uncertain. □

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Keeping science at the core

Harvey Brooks

The Education of a College President: A Memoir. By James R. Killian, Jr.
MIT Press: 1985. Pp.478. \$19.95, £19.95.

DURING the middle years of this century, James Killian presided over the transformation of the Massachusetts Institute of Technology from a highly respected engineering school to the world's leading technological university, a "university polarized around science". This last phrase is used by the author to describe an institution embracing virtually all fields of knowledge, but with each discipline in orbit, as it were, around a central nucleus of science and technology. In the same career, Killian intermingled university leadership with high-level public service, notably as science adviser to President Eisenhower and as a key figure in many seminal government advisory groups during a formative period for both science policy and defence policy. What were the experiences and personal qualities that enabled this individual, not himself either a scientist or an intellectual with academic pretensions, to become accepted as a colleague and leader by scientists and scholars, and to become an educational and scientific manager of the first rank? This highly personal, rambling and optimistic memoir does provide some insight into the answers, though it is as interesting for what it leaves out as for what it includes.

A man of broad general culture, boundless intellectual curiosity and catholic interests, very "people oriented": though not remarkably gregarious, Killian became dedicated through an unusually varied apprenticeship to "developing creative integration and interdisciplinary congeniality among a variety of research fields", and he applied an extraordinary talent for consensus-building among diverse and strong-minded individuals in both academic and political settings. He was obviously a good listener with a capacity for extracting a practical synthesis out of the ideas of creative people. Not an obvious originator himself, and lacking a strong pride in authorship, he nonetheless had a talent for recognizing people with original ideas and enabling them to realize their visions.

His success in these endeavours was reinforced by what he describes as his "clinging to a melioristic view that our future is best served by an acceptance of what Sir

Peter Medawar has aptly termed 'the hope of progress' ". This attitude, he felt, came from his "having lived so long in the regenerative sanctuary of a research university" with a "singular fellowship of young men and women working in a contagious atmosphere of excellence, discovery, and high spirits" (p. 415). He had a knack for

persuade the disciplines to subordinate their parochial interests and claims to academic "turf". Thus he sees MIT as a federal rather than as a feudal system. I suspect one could find a good many people at MIT who would not fully share this view, but I think it is, on average, correct.

Killian cites two revealing anecdotes to illustrate the source of his view of the key importance of consensus-building. Each involved the eventual failure or compromise of a good idea because of a lack of consultation with powerful interests in advance of a public commitment. The first example is an academic one. In 1956, at a public dinner in New York, he announced MIT's intention to establish within its structure a new School of Advanced Study before he had spoken to the appropriate faculty committees or the faculty as a whole. From the incident he drew a lasting lesson that "no new programs or buildings large in costs should be undertaken, however innovative, without the knowledge and advice of appropriate faculty groups" (p.138). In this instance, what he still obviously feels was an excellent idea had to be abandoned.

A parallel example on the national stage occurred soon after Killian had become science adviser to Eisenhower. In 1958, on the basis of the technical findings of the President's Science Advisory Committee, communicated to him by Killian, Eisenhower wrote to Khrushchev suggesting that a comprehensive nuclear test ban agreement was negotiable, and proposing the convening of a panel of technical experts to agree upon the design of an international detection system capable of ensuring against clandestine testing — especially underground testing — by any nation. The President's initiative, however, was taken without internal consultation with the Department of Defense, The Atomic Energy Commission or the Central Intelligence Agency.

Of the technical discussions, led on the American side by Dr James B. Fisk, President of Bell Laboratories, Killian says: "Dr. Fisk and I agreed that at no subsequent negotiation with the Soviets on arms limitation had the Soviets expressed such willingness to accept on-site inspection" (p.281). He implies that the lack of internal consultation in the US government before the Presidential initiative "was to aggravate differences in our own government" which became a major factor in America's inability to negotiate a comprehensive test ban rather than the much more limited atmospheric test ban finally achieved under the Kennedy administration in 1963. As we now know, the



James Killian on the cover of *Newsweek*, November 18 1957.

helping brilliant — often cranky and egotistical — people to sell themselves and their ideas, and he believed this could best be accomplished through conscientious and patient consultation.

Insofar as MIT was concerned, Killian rejects Clark Kerr's pessimistic view of the modern "multiversity" impervious to structural change, the advocate of change for every part of society except itself. The MIT he knew originated and assimilated many structural improvements, including new interdisciplinary laboratories, curriculum reform, undergraduate research, a new management school, new departments in the humanities and social sciences, a joint school of health sciences and technology with Harvard, and many other institutional innovations. MIT, Killian feels, has avoided the usual "hang-ups" of the multiversity by combining a single unified faculty with a strong, but accessible, style of presidential leadership, able to

rapid evolution of a sophisticated technology for underground tests largely offset the constraints on weapons development posed by the ban on atmospheric testing, and really only served to drive nuclear weapons development underground — literally and figuratively — and out of the public eye. Whether prior consultation would have merely tied the hands of the technical negotiators rather than leading to a stronger diplomatic agreement is a matter of opinion. But again, Killian's account of this episode illustrates the high importance which he attached to the building of consensus among divergent interests as a prerequisite for action (p. 279).

Although Killian's assessment of the achievements of the modern research university is highly positive, and in many

the quality of engineering and is this in turn a consequence of deterioration in the standards of engineering education? Were the reforms of the engineering curriculum to make it more "scientific" and general, which Killian obviously endorses, implicated in the problem, as some people are now asserting? Did the American preoccupation with originality, creativity and individual competitive achievement somehow result in neglect of meticulous workmanship and attention to the more routine and mundane aspects of engineering, which are so crucial to the quality and reliability of technological products? Did the great research universities, in their pursuit of a particular cultural concept of "excellence", provide an inappropriate model for the mass of the engineering and technical professions?



James Killian (second from right) with Philip Morse, Vannevar Bush and Thomas J. Watson dedicate MIT's first major computational centre in 1957.

ways forms the *leitmotif* for his whole memoir, he does concede some shortfalls and failures; however, he makes little effort to analyse the possible reasons for them. For example, while he is proud of MIT's success in creating interdisciplinary laboratories and in fostering other kinds of creative work across conventional intellectual boundaries, he admits that MIT's efforts "have not succeeded in demonstrating how these fields necessarily interact with each other and how this diverse learning is comprehended in indivisibility" (p. 402). Somehow his "enlarged vision of the place" has not quite been achieved. Yet he never even speculates as to the reasons why this might have been so.

More seriously, he suggests that the universities cannot wholly disclaim responsibility for an unmistakable decline in the quality of everyday life as so frequently reflected in American craftsmanship, in the unreliability of too much of our technology, and in the low quality of many consumer products and their service [p. 410].

This is an intriguing statement, which is simply left hanging. In what way or in what sense are the universities responsible? Does the situation reflect a decline in

Another intriguing area of ambivalence in Killian's account of the era he describes has to do with the role of the "military-industrial complex". On the one hand, the whole memoir is full of pride in the services of MIT and its staff (including Killian himself) to the nation in matters of national security — the series of interdisciplinary studies organized by MIT in the 1950s, the creation of the Lincoln Laboratory, the accomplishments of the Instrumentation Laboratory (now divested from MIT as the Draper Laboratory), Project Whirlwind, the Servomechanisms Laboratory and so on. On the other hand, he makes frequent references to the dangers of militarization, stating in one place that "the military-industrial complex . . . is still having a profoundly dangerous impact on our government" (p. 419). Even his "hope of progress" is "tempered . . . by fear of nuclear war and of an intemperate militaristic spirit eroding the most precious values of our society" (p. 415).

These are strong words, but just who is the military-industrial complex? Many of the basic technological advances that have helped fuel the arms race have emerged from the large government-financed laboratories managed and operated by some

of our great research universities — for example, the concept of multiple independently targeted re-entry vehicles and dramatic advances in missile guidance accuracy that emerged from the MIT Instrumentation Laboratory, and the high yield-to-weight thermonuclear weapons which were developed in the two weapons laboratories of the University of California. While praising the scholars from research universities who "have been speaking with knowledge, integrity, and responsibility about nuclear issues" (p. 419), Killian never quite comes to grips with what the proper role of the research universities should be in relation to the creation of military technology or even the science underlying it. Should research universities, for example, pick and choose which governmental demands they respond to according to some collective view of their potential effect upon the arms race? What would be the implications for academic freedom of the enforcement of such a collective sense of responsibility on their individual members? To what extent would such institutional self-denial decrease the capacity of academics to speak out with real knowledge and responsible criticism in the national debates over defence policy, and leave the field to those with greater vested interests than the universities? These issues have never been posed to the universities in starker form than by the current Strategic Defence Initiative; it would have been interesting to have had Killian's views as to how his favourite institutions should come to grips with this new challenge to their role.

The sanguine tone of this memoir sometimes borders on the Pollyanna-ish. It is striking that, although there are interesting personal sketches of many of the principal actors in the development of America's post-war science and defence policies, villains are notable by their absence. Admiral Lewis Strauss of the Atomic Energy Commission is virtually the only important personality even mentioned in unfavourable terms, and even here the strictures are remarkably restrained. The vehement denunciations of the military-industrial complex are notable for their lack of specificity, and the reader is left in the dark as to which institutions and groups qualify for this pejorative label, and what sorts of behaviour take them over the line from patriotism to villainy. Lobbying of the political process on behalf of funding for particular weapons systems seems to be the only identified behaviour which is beyond the pale, yet one must also ask how these activities are to be distinguished from the various "summer studies" and high-level commissions (such as the Technological Capabilities Panel, which Killian himself chaired) which were the genesis of many new weapons systems.

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Chatting fractals and New Math

Thomas Banchoff

Mathematical People: Profiles and Interviews. Edited by Donald J. Albers and G.L. Alexanderson. *Birkhäuser*: 1985. Pp.372. \$24.95.

GEORGE Pólya died recently at the age of 97. Here was a legendary teacher whose book *How to Solve It* and famous film *Let Us Teach Guessing* inspired generations of students and teachers of mathematics. Would it not have been instructive to have had the opportunity to ask him questions about his career? In recent years, Benoit Mandelbrot's filigreed fractal patterns have burst out of the pages of journal after journal, intriguing scientist and designer alike. How would it be to interview him and get some insight into the process of creating a new kind of mathematics?

In *Mathematical People* we can enjoy conversations with these figures and two dozen more. This collection of interviews and profiles comes primarily from a very successful series of articles over the past six years in *The College Mathematics Journal* (formerly *The Two-Year College Mathematics Journal*). The project began auspiciously with an interview with Pólya on the occasion of his ninetieth birthday celebration at Stanford and continued to include some of the most interesting mathematical people of our day.

Here are stories about Persi Diaconis, who left school at 14 to travel with a professional magician, Martin Gardner who explains the origin of his hugely popular *Scientific American* column, and the immensely prolific, champion collaborator Paul Erdős. We can follow the controversial views of Morris Kline about the New Math, and compare them with the counter-suggestions of Peter Hilton and Paul Halmos. We can contrast the impressions of Richard Courant given by his co-author Herbert Robbins and by the biographer Constance Reid. And we can keep track of dozens of comments about the sometimes opposing relationships between pure and applied mathematics.

What we do not find are answers to the question: "What good is mathematics anyway?" The interviewers are for the most part mathematicians themselves and everyone takes for granted the fact that the creation and communication of mathematics is a worthwhile occupation. Moreover the primary readership for the original articles was teachers of mathematics, so questions tend to centre on different aspects of teaching as opposed to research.

There is wide variation in the depth and the nature of the interviews and profiles, and occasionally a general reader will be left behind when the discussion takes a

technical turn. There are also a few other places where one looks in vain for a follow-up to a remark in an interview. For example, what was the subject of Donald Knuth's first published article, in *MAD Magazine*? Could Albert Tucker please explain why his mentor Solomon Lefschetz had artificial hands? And if David Blackman was not conscious of discrimination all through his schooling, why was it that he only applied to black institutions when he sought his first job?

By and large, however, the interview format is quite successful. By contrast the set pieces taken from popular scientific journals, on Shiing-Shen Chern and Ron

In physical context

Ivor Grattan-Guinness

Mathematics and the Search for Knowledge. By Morris Kline. *Oxford University Press*:1985. Pp.257. \$19.95, £21.

OVER several decades, Morris Kline has built up an enviable reputation as the author of a series of popular books on mathematics which include a substantial element of historical material. The first book, *Mathematics in Western Culture* (Oxford University Press, 1953), remains perhaps the best: the last one, *Mathematics: The Loss of Certainty* (Oxford University Press, 1980), was probably the worst, with numerous infelicities and errors over the foundations of mathematics and its history. This latest volume lies somewhere between the two.

Here, Kline has returned to applied mathematics, in order to explain how mathematics helps us to understand the physical world. After a "Historical Overview" dealing with various philosophers' positions on the possible existence of an external world, and a discussion of the fallibility of sense perception, he proceeds to a chronological account of episodes in the history of astronomy and mechanics: the Greeks, Copernicus and Kepler, Descartes and Newton. Then electromagnetism is introduced, chiefly as background to relativity and quantum mechanics. The final trio of chapters contains discussions of aspects of the philosophical questions which underpin the book as a whole.

The level of success achieved by Kline in answering these questions may be appraised at first by looking at his use of history. The treatment is more detailed than in his *Mathematics and the Physical World* (Crowell, 1959), but not much; in particular, there is not actually a great deal of mathematics in the book, the chapters on general relativity and quantum mechanics including hardly any at all. Further, the range of applications is curiously incomplete: there is little or nothing on optics, heat diffusion (the paragraph on p. 202 about Fourier mis-

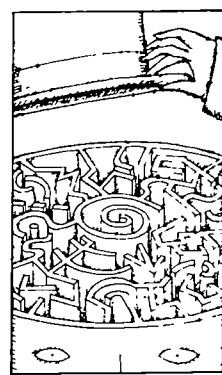
Graham for example, may be better organized and generally more informative but they lack the spontaneity of the transcribed interviews. The detailed and technical autobiographical reminiscence of Olga Taussky-Todd, originally written as part of a Caltech oral history project, seems out of place in this volume.

Still, one must expect such vagaries in a collection such as this. Many people will find illumination and entertainment in the book, and will be happy to learn that a second volume is in preparation. □

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represents his philosophy, overrates his mathematics and underrates his physics), hydrodynamics, elasticity theory or engineering mathematics (friction studies, machines of all kinds, cartography and topography, and so on). Probability and statistics earn the falsehood that "the theory of probability . . . entered into mathematics quite by chance in connection with games of chance" (p. 189): the virtual absence of these two topics is an especial pity, since work on them greatly extended the range of application of mathematics and raised important questions about knowledge and ignorance, and the epistemological status of approximate theories.

The result of these omissions is not only



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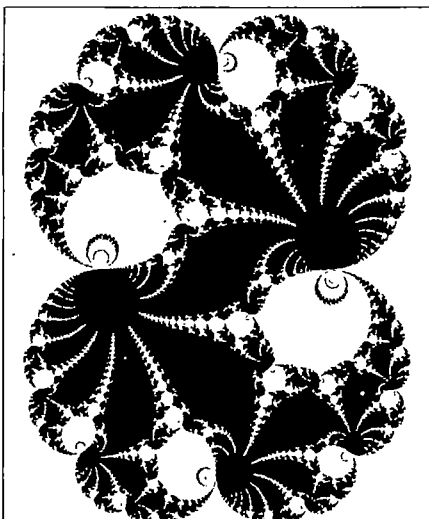
Vaulting Ambition is indeed the last word on the subject of sociobiology. It is meticulous in its argument and total in its scope. It deals a carefully reasoned blow to the pretensions of sociobiologists.' *Richard Lewontin*
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that many essential historical episodes have been passed over, but also that certain crucial aspects of the ways in which mathematics aids our search for knowledge are absent. The terms within which the subject has been tackled are too modest, a failing which also applies to the weight of supporting mathematical apparatus which is brought to bear. There is, for example, no discussion of the calculus, so that the "mathematicization" of continuous media and phenomena cannot be described properly. The clash between different mathematical styles is not



A Julia set and its interior for the map $z \rightarrow z + i|z|$. Taken from *Mathematical People* (see previous review).

brought out, especially between the geometrical (where the properties of the given configuration play a role) and the algebraic (where formal manipulations of general basic formulae are executed, with deliberate avoidance of geometrical processes); analytical and topological styles bring further differences in their train. Finally, the extent to which a mathematical expression (in any style) reflects the structure of the physical context in which it is used is a matter which should have loomed large, but it does not.

All of these questions involve issues about mathematical proofs, which receive little attention here. But proofs lie at the heart of much of the development of mathematics and thus of its role in the "search for knowledge". The posing of such questions would have served Kline's cause better than the pastiche accounts of philosophers' views on the external world which he does provide (but which have no special bearing on the use of mathematics). This book is best taken as another example of his ability to write breezy and readable surveys of the history of mathematics, and to tackle the philosophical background on the wing; as a contribution to the problem raised, however, *Mathematics and the Search for Knowledge* is merely a propaedeutic. □

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Where lies the science?

Anthony W. Clare

The Psychoanalytic Movement. By Ernest Gellner. *Paladin*: 1985. Pp.241. Pbk £3.50.

Psychoanalysis and Beyond. By Charles Rycroft. Edited by Peter Fuller. *Chatto & Windus*: 1985. Pp.310. Hbk £10.95; pbk £4.95. To be published early next year in the United States by Chicago University Press.

Psychoanalysis: Freud's Cognitive Psychology. By Matthew Hugh Erdelyi. *W.H. Freeman*: 1985. Pp.303. Hbk \$24.95, £25; pbk \$14.95, £14.95.

Decline and Fall of the Freudian Empire. By Hans Eysenck. *Viking*: 1985. Pp.224. £12.95.

THE way in which the psychoanalytic school was formed raises profound questions concerning its status as a science and suggests that it might, with benefit, be viewed as a secular religion. Certainly, one will look in vain elsewhere in science for another example of a body of theoretical knowledge linked so intrinsically, so inextricably, with the individual who first propounded it. In no other branch can one find an absence of an external arbitrator, that is to say someone outside the control of the system itself and able to pass independent comment on its claims, achievements and shortcomings. In the case of psychoanalysis, the location of the criterion of its truth is, in the words of Ernest Gellner, "determined by the ideas of the system itself; the well of truth is within the ramparts and not outside". The possession of this truth is not handed down in seminars and courses of instruction, though these play a small part, but by way of participation in the training analysis. As the apostolic succession from St Peter is handed down from the bishops to newly ordained priests, so the authentic link with Freud is maintained and continued by means of the transfer of authority from training analyst to analysand.

The religious analogy does not end there. As intellectual and rational criticism of revealed religious dogma is interpreted as a closing of the mind and heart to truth, and in no way a valid comment on the basic validity of the truth itself, so criticism of psychoanalysis is rejected on the grounds that either the critic has never been analysed, in which case he is resisting the need to be analysed (which itself is worthy of interpretation), or he has been analysed and his criticisms reflect an incomplete or prematurely terminated process, the result no doubt of fear over what might be revealed. And the religious analogy becomes uncomfortable when one examines the schisms and factions within the domain of psychoanalysis, with each school claiming to have adhered the

closest to the spirit as well as the law laid down by the supreme founder.

The question which Gibbon asked of Christianity is equally applicable to psychoanalysis: by what means did the new vision obtain so remarkable a victory? In a stylish, witty and deceptively readable book, Gellner exposes the secular religious nature of the psychoanalytic enterprise. He admits that a compelling, charismatic belief must possess more than merely the promise of succour in a plague and links with the background convictions of the age. It must engender a tension in the neophyte or potential convert. It must tease and worry him with its promise and its threat. The doctrine to be embraced must contain the promise of a salvation that is earnestly desired. There must be plausible reasons for believing its claims and also good reasons for doubting it or fearing its truth. The middle ground between acceptance and rejection must be denied and the issue of whether it is or is not true must be trans-empirical and untestable. The fact that confidence in psychoanalysis is devoid of any visible means of support hardly seems to diminish its authority: indeed, argues Gellner,

were its means of support visible, they would be subject to evaluation; and the assertion would have given hostages to fortune, and also conceded its own ordinary, human status — making its own contentions arguable, negotiable, imitable, publicly available rather than tied to a single source.

One psychoanalyst who has been particularly conscious of the contentiousness of the psychoanalytical factions is Charles Rycroft who, in so far as he identifies with any particular school, tends to align himself with the English-speaking school uniting such disparate practitioners as Winnicott, Fairbairn, Balint and Bowlby. This group, as Rycroft pointed out in an essay entitled "Psychoanalysis and Beyond", contained in the collection of his writings under the same title, repudiated any royal road to becoming an analyst even to the extent of encouraging trainees to have part of their teaching under the supervision of a member of another faction. Rycroft underwent, as far as one can tell, an orthodox Freudian analysis and in 1956 presented a paper to the British Psycho-Analytical Society which challenged the prevailing theory of symbolism by suggesting that it is not a regressive or defensive phenomenon but rather "a general capacity of the mind" which could be deployed in manifold different ways. According to Peter Fuller, the editor of the book, the Society received this modification, the first serious one advanced to a theory of some 40 years standing, with reserve while it struggled to protect the orthodox position. It was the first of a number of theoretically awkward questions that Rycroft was to raise, on such issues as dreams, fantasies and the nature of psychoanalysis itself.

In 1968, he wrote *A Critical Dictionary*



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of *Psychoanalysis*, a task which enabled him to take all the current psychoanalytical theories and systems to pieces and compelled him to construct some kind of overview embracing the ideas of Freud and the British analysts — such as Anna Freud, Melanie Klein, Michael Balint and Donald Winnicott — who had been active when he himself had been a student. His growing conviction that psychoanalysis is not so much concerned with the causes of human behaviour as with its meaning, and is better seen as one of the humanities than a science, does enable him to side-step many of the objections to psychoanalysis made by those such as Gellner and, as we shall see, Eysenck. However, nowhere in the 25 essays contained in this collection does he apply his mind to the issue which clearly troubles critics, namely the extent to which psychoanalysis is a self-fulfilling explanatory theory, whether it be of meaning or causes.

Meanwhile, there are others such as Professor Erdelyi, of the Department of Psychology at Brooklyn College, who struggle to salvage as much of Freud's expansive theory as modern insights in cognitive, physiological and neurological psychology will permit. Erdelyi's book uses case histories, laboratory studies, newspaper cuttings, visual art and poetry in an imaginative if occasionally wayward examination of Freudian theory. What emerges, however, suggests that what was lasting in Freud's conceptions is their general preoccupations. The specific elements of that gigantic canvas — the separation of mental functioning into id, ego, superego, theories of dream function and the nature of repression — just do not stand up to the relentless scrutiny of the laboratory scientists or the critical clinician. A particularly good example concerns Freud's pleasure principle. Freud assumed that pleasure/positive reinforcement resulted from tension-drive reduction; that, in short, pleasure was the cessation of pain — the so-called "Nirvana principle". The physiological facts suggest

otherwise. Pleasure and unpleasure appear to be not opposite sides of the same coin but two different coins altogether. More uncomfortable for psychoanalysts is the fact that the "pleasure" arising from stimulation of the pleasure centre in the brain is general in nature and *not* sexual as a great deal of psychoanalytical writing and excavation argues.

The most fundamental criticism of psychoanalysis, however, remains the same, 30 years on from when Hans Eysenck first mounted it. It is the fact that there is no convincing evidence that patients do better under analyses than they do under some other form of psychotherapy or psychiatric treatment. Such criticism does not come only from without. Anthony Storr, himself a Jungian analyst, has concluded that "the evidence that psychoanalysis cures anyone of anything is so shaky as to be practically non-existent". And Brian Farrell, a philosopher by no means hostile to analysis, has admitted that the impact of psychoanalysis

cannot be justified on the grounds that it contains a body of reasonably secure or established knowledge . . . it [is] only too painfully evident that analysis does not contain any such body of knowledge.

Characteristically, Eysenck puts the case with more exuberance and venom. At best, he concludes his devastating polemical assault, psychoanalysis is "a premature crystallization of spurious orthodoxies; at worst a pseudo-scientific doctrine that has done untold harm to psychology and psychiatry alike". The final verdict, however, is not in and it is doubtful that it will be within the lifetimes of the authors of these books. What does seem unarguable, however, is that in the one hundred years that have passed since Freud began to build his theoretical framework the theory still lacks any substantial validity and his school remains beleaguered. □

Anthony W. Clare is Professor in the Department of Psychological Medicine, St Bartholomew's Hospital Medical College, West Smithfield, London EC1A 7BE, UK.

Scent of semi-science

Steve Blinkhorn

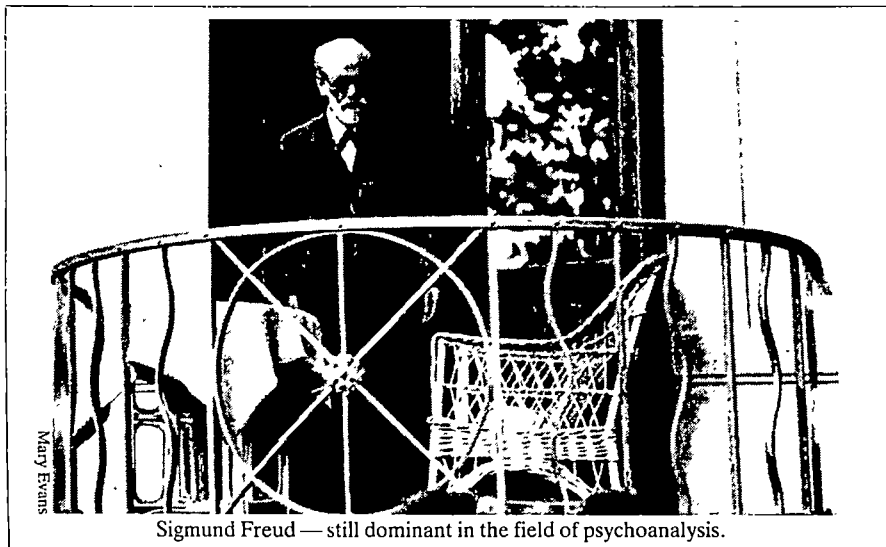
The Intelligence Men: Makers of the IQ Controversy. By Richard E. Fancher. W. W. Norton: 1985. Pp.269. \$17.95. £14.95

AT LAST, it seems, the soot and whitewash school of controversy has had its day. Here is a book about the IQ debate which is at once absorbing, informative, deftly written and, if the expression can be pardoned, a thundering good read.

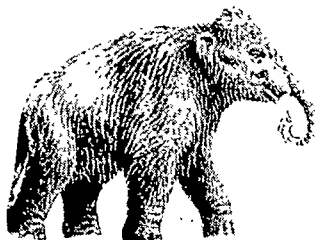
The Intelligence Men is that rare inversion of the natural order of things: a first-rate book based on a second-rate notion. The notion, that one may discover why particular figures in the history of a debate took up their respective positions by examining their personal histories, is only as good as the sample of biographical material available and the accuracy with which it is interpreted; in any case, the standards of proof required of such a notion are far inferior to those proper to the debate itself. Fortunately analysis of this sort intrudes very little into Fancher's account of the lives and work of a dozen or so major contributors to the long-running dispute over IQ.

The delicious consequence of this plan has been a book reeking of the dubious scent of semi-science in the making. Where often a scientist is known to a wider public only as an author of published work, here that work is set in a personal context which illuminates and informs even when the technical level of the narrative is less than satisfactory. Pasteboard textbook characters have pasteboard theories to peddle. But the facts that Spearman registered for a PhD with Wundt (and took seven years to complete it), that Terman's introduction to psychology was a book on phrenology and that amongst Yerkes's motivations for seeking a career as a physician was the fact that it paid better than being a farmer, bring an unusual sparkle to academic history.

Kamin's brush with McCarthy's subcommittee on unAmerican activities; Jensen's origins as the son of a lumber and building-supplies dealer, and habit of conducting recorded symphonies at home with a chopstick for a baton; Binet as a child being forced by his father to touch a cadaver; Burt labouring to devise raw data to fit published statistics — none of this is relevant to the logic of scientific discovery. But, as Fancher suggests, the psychology of the scientist is at least as potent a force in determining the direction of theory. This, of course, is a view that can be taken to extremes. There was once a claim that Spearman, being British and therefore a monarchist, was driven to expect a single factor of intelligence, whereas Thurstone, being American and more democratically inclined, preferred a federalist view with several distinct abili-



Sigmund Freud — still dominant in the field of psychoanalysis.



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ties ranking equally. Nothing so crude enters Fancher's book; rather, there is an elusive subtlety to the drift of the narrative that no review can hope to capture.

It is all the more unfortunate, then, that the last chapter reads like a hurried afterthought. In attempting to come to a balanced view of the current state of the debate, the author steps outside his role of historian and immediately becomes unconvincing. This is a pity, but a small blemish. As the origins of intelligence testing recede beyond living memory, this book does a real service by reminding us that present academic fisticuffs have their origins in an honest but often naive and blinkered struggle to make sense of human variability. No doubt future historians will judge present efforts to have been equally blinkered; but in what proportions are we now naive and honest?

Steve Blinkhorn is Director of the Psychometric Research Unit and Principal Lecturer in the Department of Psychology, Hatfield Polytechnic, PO Box 109, College Lane, Hatfield, Hertfordshire AL10 9AB, UK.

Go for Valhalla

Michael Spencer

The Joy of Science: Excellence and its Rewards. By Carl Sindermann. Plenum: 1985. Pp.259. \$16.95, £16.15.

SAMUEL Smiles, the Victorian author of such uplifting texts as *Self-Help* and *Men of Invention and Industry*, has been enjoying a revival of esteem on the New Right. Britain's Prime Minister has told unemployed teenagers to go out and start their own businesses, while one of her ministers has coined the immortal phrase "Get on yer Bike". We are urged to model ourselves on those who pursue success with relentless enterprise and determination.

Carl J. Sindermann is the Samuel Smiles of science, and has followed his earlier book *Winning the Games Scientists Play* with a further guide on How to Get On. His technique is modelled on that of Smiles: to examine in detail the upward climb of those who have achieved success. By "success" he means not the winning of Nobel prizes (a chimera that motivates more scientists than would ever admit it), but a job-satisfaction extending to administration and the manipulation of grant-giving bodies.

He cheerfully admits in his preface that the prospect of such a book did not endear him to his colleagues, who may have suspected that their own careers (thinly disguised under fictitious names) would be dissected for all to see. Undaunted, at late evening cocktail parties and professional meetings he pursued his chosen paragons long after their normal bedtimes, so as to analyse what made them tick.

The chapter on "The Ascendant Female Scientist" was, says the author, written against the strongly stated advice of all his female acquaintances, who were still smarting from a section entitled "Sex in the Laboratory" in his previous book. None of them, he says, would admit to the classification of "friend" after it was published.

Why, then he do it? The answer appears to be that Dr Sindermann is an old-fashioned scientific optimist who believes that all science is exciting and wonderful, and that anyone who succeeds in it is to be applauded and emulated. (In this he may have something in common with the august figure who once opined in print that it would be a pity for the world to blow itself up with nuclear weapons because that would halt the onward march of science.) Hardly anywhere in the book — whether discussing choice of research topic, career transitions or interactions with the outside world — does he discuss the problem of deciding whether a project is socially desirable. The word "ethics" does not appear in the index.

Dr Sindermann has little time for the also-rans, except as examples of where one can go wrong. He would certainly not agree with a friend of mine, who emerged limply from a two-hour session with a visiting celebrity to remark that the failures were much more interesting to talk to. He has harsh words to say about the Burnout, the Fade-out and the Guaranteed Loser. The latter category includes the Unwary Activist, who may (he says) damage his or her scientific credibility by espousing causes for social reform. The author classes such misguided people along with the Dilettante, the Hobbyist and the Chronic Underachiever.

Since most of us (by definition of that uncompromising word "excellence") are not going to reach Dr Sindermann's Valhalla of success, why should anyone read his book? The upwardly mobile graduate student with a taste for ruthless self-advancement will have his own ideas on how to make it. For those who have reached what the author calls the "midlife crisis" it is probably too late. The ageing scientist (to whom a chapter is devoted) is not going to believe a word of it.

Perhaps the most interesting thing about the book is what is missing from it. There is the same moral vacuum in the Royal Society's message to scientists, that they should learn how to manage the media in support of their chosen way of life. It is possible — just possible — that the current public mood of suspicion and alarm about the outcome of some scientific research is due not to inadequate public relations, but to a failure among scientists to think about the repercussions on ordinary people. Politicians have made the same mistake. □

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Modularity of brain and mind

P.N. Johnson-Laird

The Social Brain: Discovering the Networks of the Mind. By Michael S. Gazzaniga. Basic Books:1985. Pp.240. \$17.95.
Neuronal Man: The Biology of Mind. By Jean-Pierre Changeux. Translated by Laurence Garey. Pantheon:1985. Pp.348. \$19.95.

THE brain is the organ of the mind. If you want to understand the mind, then you had better understand the brain. These two books, not perhaps as different as chalk and cheese, but certainly as different as brie and parmesan, share this philosophy. They both aim to elucidate the mind by establishing how the brain works. Michael Gazzaniga wants to demonstrate a link between the global organization of your brain and the way in which you come to believe in certain propositions — from prosaic assumptions about yourself to your propensity to hold religious beliefs. Jean-Pierre Changeux is a Gallic ghost-buster, who aims to rid you of your faith in the "ghost in the machine". He will explain your mind away: it is nothing but neurones, synapses, and electrical and chemical signals. His motto is *La Mettrie*'s: "The soul is merely a vain term of which we have no idea. Let us conclude boldly that man is a machine".

Gazzaniga is a distinguished neuropsychologist at the Cornell University Medical Center in New York, and is best known for his studies of the psychological consequences of the "split-brain" operation. His book is an intellectual autobiography that recounts the development of these studies, considers their implications and speculates about such larger issues as society, prehistory and religion. He writes in a loose, unbuttoned style, with numerous asides and anecdotes, and is at his strongest in describing his beautiful series of experiments on the effects of the split-brain operation.

If you fixate some point in the scene in front of you, then everything to the left of your fixation point is projected to the right half of your brain, and everything to the right of your fixation point is projected to the left half. Information is rapidly transferred from one half to the other by a massive bundle of nerve fibres, the corpus callosum. The split-brain operation is carried out to control extreme forms of epilepsy. The surgeon cuts the corpus callosum and its associated structures so as to isolate the left cerebral cortex from the right. The operation is highly beneficial in otherwise intractable cases, and, according to early results, had no effects on personality, mood or behaviour. However the absence of behavioural consequences did not square with some earlier studies on

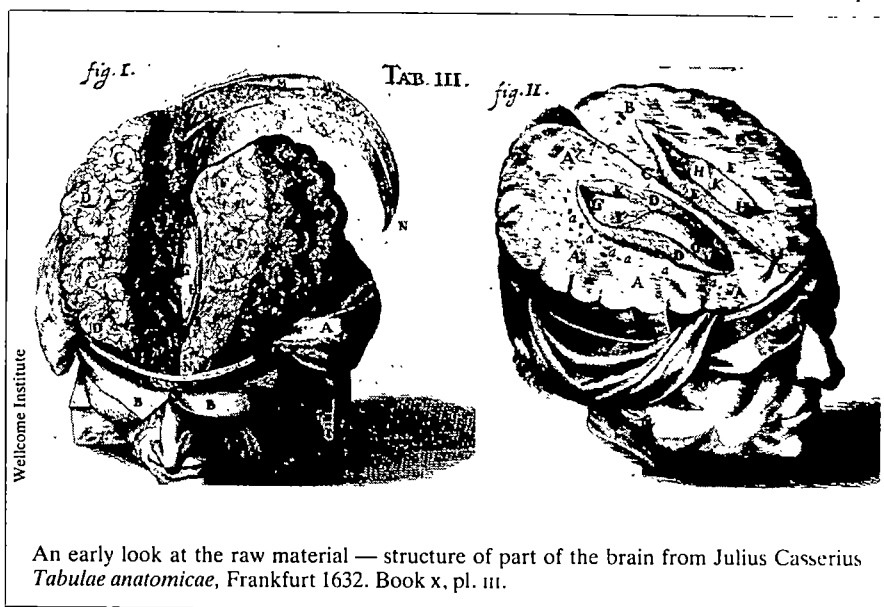
animals carried out by Roger Sperry, and so Gazzaniga decided to check again. Under Sperry's supervision, he carried out the pioneering series of studies that revealed the operation's consequences.

The first effect he observed was the patients' inability to name anything that lies in the left visual field: such a stimulus projects to the right hemisphere, but the main language centres are located in the left hemisphere. Information can no longer be transmitted from one hemisphere to the other, and so the patients deny seeing the stimulus. However, when they are asked to make guesses about it, their emotional evaluations are almost identical to those they make when they can see it. They become frightened by terrifying pictures, though they still deny seeing them, and may attribute the cause

such distractions and thus scores higher on perceptual tasks.

When split-brain patients are given two matching tasks, one to solve in the left brain and the other to solve in the right brain, they readily perform them, but the verbal justifications they give for the responses made by the right brain are usually false even though the responses themselves are correct. Thus, if the command "walk" is flashed to the right brain, then the patient will indeed get up and start to walk. If asked why, the left brain, which is unaware of the command, readily concocts a confabulatory explanation. Here is the seed for Gazzaniga's principal hypothesis.

The brain is organized, he claims, into separate modules that can carry out various actions, maintain moods and per-



An early look at the raw material — structure of part of the brain from Julius Casserius *Tabulae anatomicae*, Frankfurt 1632. Book x, pl. iii.

of their fear to the demeanour of the experimenter.

If I remove the cooling fan from my micro-computer, the machine is soon incapable of carrying out any computations. Someone who knows little about computers might therefore infer that the fan plays a central role in computation. Analogous errors are an ever-present danger in the study of brain damage. Gazzaniga wisely resists them and repudiates the one-time vogue for alleged dichotomies between left-brain thinking (analytical and verbal) and right-brain thinking (intuitive and visual). His further studies have revealed some subtle phenomena. The right hemisphere typically can create better drawings than the left. Yet the right is not necessarily a superior visualizer: it does no better than the left in matching one readily nameable picture to another. Gazzaniga suggests that perhaps the right brain does not possess a special ability at perception. Instead, if the left brain has no immediate opportunity to exercise its linguistic ability, it may concentrate still more on the verbal aspects of the task — to the detriment of its perceptual performance — whereas the right hemisphere has no

ceive the world. They are independent units that work in parallel; and they contribute to what we are conscious of, but we are not aware of them. They can control behaviour, and so we sometimes do things for no reason that we are aware of — we act capriciously. The module that mediates language and consciousness, however, interprets our behaviour, and instantly contrives theories to explain it. That is why we think we have free will, and why we are likely to hold beliefs for which we have no objective evidence.

Another inherent danger in interpreting brain damage is to overlook the possibility that its effects are a result of compensatory changes. A sceptic might accordingly argue that Gazzaniga is right: one half of a split brain does contrive explanations for how the other half lives, but only because it has been forced to do so as a result of the operation. Normal people behave quite differently and are conscious of the roots of their behaviour. Of course, there is a long tradition in psychology — and considerable evidence — to the contrary. Likewise, the modular hypothesis has been independently postulated by a number of cognitive scientists in order to

make sense of the mechanisms of vision and language, the distributions of scores on different sorts of mental test, and other neurological disturbances. What is unique in Gazzaniga's account, however, is his emphasis on the confabulatory capacity of the interpretative module.

Knowledge of the brain has changed strikingly in the past 25 years. It used to be assumed that sensory information was conducted to the cortex, where there was an interchange with other information in the so-called "association" areas, and finally neuronal signals were transmitted to the motor areas responsible for the control of actions. This picture is now known to be a vast oversimplification. Jean-Pierre Changeux, who is a molecular neurobiologist at the Collège de France, has made important contributions to these developments in his work on neurotransmitters and the growth of neural networks. His prize-winning book was evidently a best-seller in France, and it is an outstanding attempt to convey to the general public an interdisciplinary understanding of the human nervous system.

Changeux begins with the tortuous history of concepts of the brain. He records the gradual recovery from Aristotle's original blunder in assuming that it functioned as a cooling device — a case of confusing the central processor with the fan — and describes the gradual ousting of the idea of "vital forces" by the modern electrochemical theory of the propagation of nerve impulses. The brain contains a dense interlacing of the dendritic webs of thousands of millions of neurones, through which pass myriads of electrical impulses, relayed across the synapses from one neurone to another by chemical transmitters or in some cases electrically. There are dozens of chemical substances that are now known to function as neurotransmitters, or as modifiers of their actions, including the nervous system's own internal pain-killers, the enkephalins and endorphins. The principles of the "wiring" of the nervous system are the same throughout the cortex, regardless of the functional specialization of a particular area. Nature seems to use the same basic building blocks over and over again, and employs no cells, circuitry or neurotransmitters unique to human beings. Organization determines function, and the modular principle extends downwards from regions of the brain that mediate

major functions to the cortical columns of neuronal structures. Changeux describes these discoveries with clarity and panache, making excursions into the brain chemistry of pain, thirst, rage and even orgasm.

Behaviour can be explained in terms of the mobilization of sets of nerve cells; their responses can be explained in physicochemical terms; and these interactions can be explained at the molecular level. But what about the subjective life of the mind, such as our ability to imagine a friend's face, our emotional experiences and our sense of self-awareness? Changeux's thesis is that mental states are identical to physical states of the brain. Percepts, images, concepts and all such "mental objects" correspond to the activity, electrical and chemical, of assemblies of neurones, dispersed through separate regions of the brain in the case of abstract ideas. Changeux admits that a given mental object may be constructed from slightly different neuronal populations, which may even differ in detail within the same individual from one moment to another. But he does not deal adequately with how the same mental state could arise from such different physical states of the brain.

The answer to this problem is to be found in a different species of materialism that can be traced back to Kenneth Craik and Alan Turing. What mentality depends on is not a particular physical substrate, but the functional organization of the processes that it makes possible. There is still no need to invoke mystical properties in explaining the mind, but this approach can be informed by the theory of computability. The brain is clearly very unlike an ordinary digital computer in the details of its construction and operation; yet it is arguably a computational device that is causally connected to the external world.

If one thinks of the brain as a system containing many processors that carry out computations in a parallel and distributed way, one can make sense both of Gazzaniga's modular hypothesis and of the relation between mentality and neurones — just as different algorithms can compute the same function, so the same mental state can arise from different configurations of neurones provided that the functional organization of their processes is the same. It is therefore necessary to understand what the mind's various computational tasks are, how they might best be carried out, and how such procedures can be neurally embodied. It is a pity that the work of Craik, Turing and the late David Marr, who did so much to clarify these issues, lies outside the scope of these two books. The brain is the organ of the mind, but the dependence cuts both ways. If you want to understand the brain, you had better understand the mind. □

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Entropy parameters and quantum jumps

Walter Gratzer

Mayonnaise and the Origin of Life: Thoughts of Minds and Molecules. By Harold J. Morowitz. Charles Scribner's Sons: 1985. Pp.256. \$15.95.

Natural Acts: A Sidelong View of Science and Nature. By David Quammen. Lyons Books (Schocken): 1985. Pp.221. \$16.95.

THE first of Dr Morowitz's essays finds him on the Galapagos Islands, the third in an aeroplane approaching New Delhi and not long after we meet him pacing the deck of a cruise boat on Glacier Bay in Alaska. Where other orbiting professors sort their slides or touch up the odd grant application, Dr. Morowitz, it seems, reaches for his pen and kicks his muse into action.

As a popularizer of science Morowitz is by no means in the Haldane class, but, at his best, as when he is ruminating on the quirks of scientists, dead and alive, and the assaults on science by the Great Unwashed, he is distinctly good company. He writes captivatingly on the eccentric palaeontologist, John Bell Hatcher, and on the unhappy Philip Gosse, a Victorian worthy, who as a Plymouth Brother, a zoologist and FRS, and friend of Darwin, was tormented by the geological and palaeontological evidence against Bishop Ussher's chronology and by such teasers as whether the occupants of the Garden of Eden possessed navels. (Such matters were far more satisfyingly resolved by the mediaeval schoolmen: what could be more pleasing, for example, than the logic of Origen, who held that since the sphere was the most perfect of all shapes by virtue of the invariant distance between its centre and all points on the surface, we would all on the Day of Judgement be transformed into spheres and roll into paradise.) Morowitz respects men such as Gosse for their tortured honesty of intellect. He deals sharply, by contrast, with those modern fleas on the body of science, the creationists and parapsychologists. One of his most entertaining pieces is a magisterial analysis of the thermodynamics of ESP.

I am less sure about a stertorous attempt to rehabilitate Teilhard de Chardin. But then again Morowitz comes up trumps on popular perceptions of entropy, which seems to have joined parameter, extrapolate and quantum-jump in the journalistic vocabulary. Here is a political pundit, quoted by Morowitz: "Entropy helps to explain why we have runaway inflation, soaring unemployment, bloated bureaucracies, a widely escalating energy crisis and worsening pollution". Poor Boltzmann (over whose remains in Vienna is hewn in stone the inscription

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J.C.J. Nihoul, Liège University, Belgium (editor)

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B. Velde, E.N.S., Laboratoire de Géologie, Paris, France

(Developments in Sedimentology, 40)

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Editors: A. Thiede, E. Deltz, R. Engemann, H. Hamelmann, University of Kiel

1985. 145 figures, 76 tables. XIX, 392 pages. Hard cover DM 198,- ISBN 3-540-13221-X

Several international research groups have contributed their work and experience to the compilation of this comprehensive survey of microsurgical models of organ transplantation in the rat. It demonstrates operative techniques of all types in detail and gives the results of immunological and morphological analyses of rat organ transplantations. Not only a wide range of organs possible for operations, but also a variety of experimental models for the analysis of problems arising from organ transplantations are presented.

Glucocorticoid Hormone

Mechanisms of Action

Editors: Y. Sakamoto, F. Isohashi, Osaka University

1985. Approx. 230 pages. Hard cover DM 69,- ISBN 3-540-15583-X Distribution rights for Japan: Japan Scientific Societies Press, Tokyo

This book covers the latest advances in glucocorticoid hormone receptors and the molecular mechanisms by which these hormones exert specific effects in target tissues. The underlying physiological process is divided into three steps: a steroid-binding step, an activation or transformation step, and a translocation step, and the first chapters in this book are arranged accordingly. Later chapters are devoted to recent developments in other steroid receptor systems and the mechanisms of glucocorticoid resistance. Each chapter is an invited contribution by an internationally recognized scientist and includes previously unpublished results as well as up-to-date references.

First Trimester Fetal Diagnosis

An International Symposium

Convento delle Clarisse, Rapallo, Italy. October 25-27, 1984

Editors: M. Fraccaro, Pavia; G. Simoni, B. Brambati, Milano

1985. 133 figures. IX, 355 pages. Hard cover DM 168,- ISBN 3-540-15785-9

This is a new and comprehensive study of all aspects of first trimester fetal diagnosis of genetical diseases. It describes new obstetrics, cytogenic, biochemical and molecular methods based on the use of chorionic villi sampling, and the pioneers and initiators of these methods discuss the different approaches to trophoblast. The book also reports on direct and culture methods for chromosome preparations, biochemical experiments, DNA studies and fetal pathology, and offers an evaluation of the diagnostic results and risks so far discovered worldwide.

U.F. Westphal, Annapolis

Steroid-Protein Interactions II

1985. 80 figures, 153 tables. Approx. 620 pages. (Monographs on Endocrinology, Volume 27). Hard cover DM 320,- ISBN 3-540-15321-7

Since volume I of the monograph was published in 1971, significant developments in the study of steroids have demanded a completely new edition. This book is both a supplement and continuation, following up previous discussions and shedding new light on steroid interaction with serum albumin and α_1 -acid glycoprotein. It greatly expands on topics of anti-steroid antibodies and steroid transforming enzymes, and reports mainly on progress in corticosteroid-binding globulin (CBG), progesterone-binding globulin (PBG), and sex steroid-binding protein (SBP).

Peptide Hormones in Lung-Cancer

Editors: K. Havemann, Marburg; G. Sorenson, Hanover, NH; C. Gropp, Wuppertal

1985. 100 figures, 63 tables. XII, 248 pages. (Recent Results in Cancer Research, Volume 99). Hard cover DM 138,- ISBN 3-540-15504-X

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Tumor Aneuploidy

Editors: T. Büchner, C.D. Bloomfield, W. Hiddemann, D.K. Hossfeld, J. Schumann

1985. 49 figures, 56 tables, 152 pages. Soft cover DM 48,- ISBN 3-540-15376-4

From the preface: "The contributions collected in this book cover several major aspects of present knowledge concerning the occurrence and clinical significance of chromosome abnormalities as delineated by karyotype analyses or measurements of the cellular DNA content. Updated results and surveys are contributed by authors from 8 centers in Europe and in the United States. As a result of their cooperation this volume presents an overview of the current status of cytogenetics in human malignancies with particular emphasis on their clinical relevance."

P.L. Ballard, University of California, San Francisco

Hormones and Lung Maturation

With a contribution by R.A. Ballard

1985. 85 figures, 80 tables. Approx. 370 pages. (Monographs on Endocrinology, Volume 28). Hard cover DM 198,- ISBN 3-540-15320-9

Contents: Lung Development. - Glucocorticoid Effects in Vivo. - Glucocorticoid Receptor. - Role of Endogenous Corticosteroids. - Antenatal Glucocorticoid Therapy: Clinical Effects. - Antenatal Glucocorticoid Therapy: Hormone Concentrations. - Thyroid Hormones: Effects and Binding. - Beta-Adrenergic Agonists. - Other Hormones. - Hormone Interactions.

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$S=k \log \Omega$) surely did not foresee this.

Morowitz offers some agreeable literary diversions and his thermodynamite's view of the passage in *Ulysses*, in which Leopold Bloom boils a kettle for his shaving water, deserves to be preserved. He is also rather good on dentists; he enjoys the "intellectual and aesthetic pleasure" of feeling a craftsman at work in his mouth. (Bernard Shaw took the same view: there was nothing more soothing, he wrote in one of his music criticisms, after a harrowing series of recitals, than to have one's teeth drilled by a finely-skilled hand.) There is much in this collection to entertain, sometimes to annoy and only occasionally to bore.

David Quammen disarmingly confesses to being a dilettante rather than a scientist, "a haunter of libraries and a snooper". He writes natural history in an unmistakably American style and is given to apostrophizing his reader in sporting metaphors. "Truly", he says, speaking of the larger kind of sea cucumber, "these guys are out in left field". This, I own, stretches my tenuous grasp of baseball. And what, for that matter, is this Hostess Twinkie, that a seal allegedly resembles in section?

The sea cucumber opens Quammen's collection, and its trials are considerable: the tiny parasitic *candiru* fish, for instance, enters and leaves the cucumber with impunity by way of the nether passage, lingering only to feed on its vitals. "You'd think", Quammen exclaims, "the little bugger was selling encyclopaedias". Well, these are not the lapidary periods of a Medawar or Gould; but we do learn how the resourceful cucumbers deal with the situation when they eventually weary of the tiresome intruder: they evert themselves and "blast their own gut and internal organs out through their own anus". Humans are denied this recourse, and, says Quammen, when the *candiru* enters the urethra it lodges there, its spines facing backwards, and the only alternative to death by blood poisoning is amputation. Beginning to feel faint, I passed on to the next story, which is about bats, and here was rewarded by an account of a scheme by the American military during the Second World War to subdue the Japanese by dropping bats, each fitted with a napalm bomb and a little parachute, on their cities.

Quammen's book is full of strange information about animals and their habitat, about Tycho Brahe's artificial nose, and other such oddities. His exuberance carries one, sometimes unwillingly, along. He has a penchant for the poetry of Donne, and an informed concern for wildlife and the environment that is altogether heartening; his book deserves every success. □

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Behind the scenes in the fight on tropical diseases

Adetokunbo O. Lucas

From the Face of the Earth*. By June Goodfield. André Deutsch: 1985. Pp. 256. £10.95.

THE public exhibit an insatiable hunger for news about medicine and the medical sciences. In this book June Goodfield provides a five-course offering to feed the layman's appetite for "stories of medical achievement". As popular science, it translates into everyday language scientific information that is often obscured by jargon. The story telling is enlivened by taking the reader behind the scenes of scientific events — we read not only of dedication and the determination to succeed, but of personal disputes, divorce, dementia and other such human problems.

The first story about Kuru contains all the excitement of a strange disease, uniformly fatal among the sufferers, with a maximal impact on women and children and threatening to wipe out a whole community. This account highlights the role of those who finally solved the mystery. At the top of the list is Carleton Gajdusek, the deserved winner of a Nobel prize for identifying the cause of Kuru and for the discovery of slow viruses as causes of human disease. June Goodfield, however, notes but does not stress the point that control of Kuru in Papua New Guinea did not result directly from the outstanding biomedical and behavioural research which she so vividly describes. Rather, the decline in the incidence of Kuru apparently stemmed from action taken by some person(s) unnamed, who, in the 1950s, forced a cultural change in the ritual method of disposing of the dead. Who,

one wonders, were these faceless persons? What was their motive? Was it mere abhorrence of a strange cultural practice, or did they somehow intuitively sense that such practices could have fatal consequences?

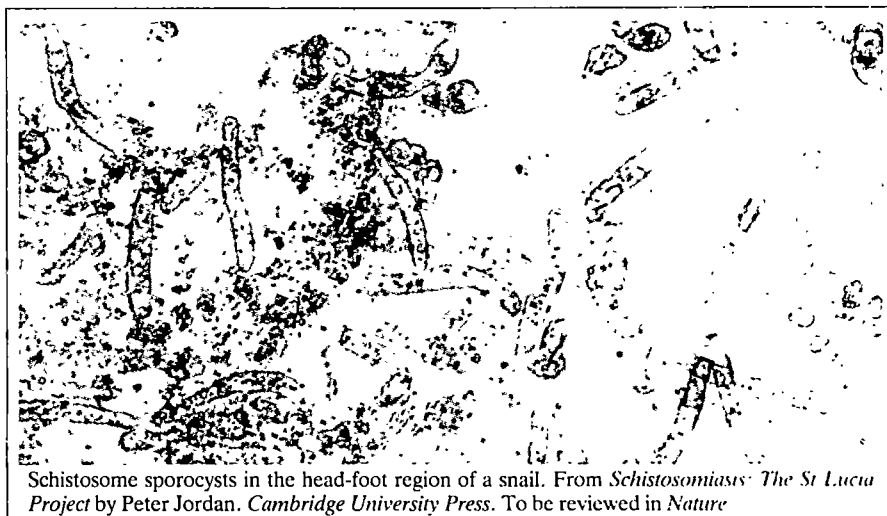
The Kuru story illustrates the fall-out effect by which research on a specific problem can generate new knowledge of fundamental importance and unexpected benefit in other areas. Thus the work in Papua New Guinea has brought to Europe and North America new insights to diseases of local interest, such as Gerstmann-Straussler Syndrome and Creutzfeldt-Jakob Disease.

Discipline, dedication and imaginative resourcefulness are the main ingredients of the next story on hepatitis B vaccine. Illustrated here is the point that the identification of the appropriate risk groups is an essential stock-in-trade of the epidemiologist, from classical studies on scurvy among English sailors, to research into cancer of the lung among smokers.

"The Three Valleys of St Lucia" deals with a large operational research programme aimed at comparing strategies for the control of schistosomiasis. Unlike Kuru, a unique disease in an island population, schistosomiasis is widespread in the tropics, affecting some 200 million people. It is not new — its eggs have been found in an Egyptian mummy — but it presents a fresh challenge where projects such as irrigation schemes and man-made lakes result in expansion of the population of the vector snails, an increase in man-water contacts, and thus intensification and broadening of the distribution of the disease.

The St Lucia story well illustrates the practical questions that need to be answered in translating theoretical options into effective strategies. And, again, the research provided answers that were useful not only on St Lucia but also in other areas. Recently, the World Health Organization proposed new strategies for the control of schistosomiasis which owe much to the findings at St Lucia, updated to take note of recent advances in technol-

*In the United States *Quest for the Killers*, published by Birkhäuser price \$24.95.



Schistosome sporocysts in the head-foot region of a snail. From *Schistosomiasis: The St Lucia Project* by Peter Jordan. Cambridge University Press. To be reviewed in *Nature*

ogy, in particular the availability of a new, well-tolerated drug.

The chapter on leprosy is interesting, but perhaps premature in comparison with the other stories. It tells of the search for a vaccine; but while much progress has been made over the past decade we are still far from finding a vaccine of proven efficacy for operational use.

The last story, on the fight against smallpox, provides a grand finale in that it fully justifies the title of the British edition of the book. This was a unique achievement, the total eradication of a disease that presented a worldwide challenge. Even though Kuru may disappear, one cannot sign its death certificate with the same confidence as was done for smallpox on 8 May 1980 at the World Health Assembly in Geneva. Although there has been some speculation about the feasibility of completely eradicating other infections "from the face of the Earth" — proposed candidates include measles and poliomyelitis — no other disease presents the combination of features which made possible the difficult task of eradicating smallpox.

Most of the data required for epidemiological surveillance of smallpox could be obtained from fairly simple clinical observations of sick patients, and the examination of scars of healed natural infections or vaccination scars. Laboratory help was required in the identification of the small proportion of atypical cases, and for the differentiation of smallpox from other pox diseases. Most other infections present more formidable problems; the clinical features are usually not sufficiently characteristic to permit differential diagnosis on simple clinical examination, and there are usually no pathognomonic scars to identify those who have had previous infections or are protected by immunization.

Apart from satisfying the demand for exciting accounts of medical discoveries, this book and similar ones draw the public into the debate about investment in the biomedical sciences. On the one hand, at times of economic recession, reduction in the budgets for biomedical research often seems a soft option because the venture does not command a powerful constituency. On the other hand, when threatened by the appearance of such problems as Legionnaire's disease and AIDS, people are anxious to know that something is being done to control the scourge. This book shows that breakthroughs do not leap out on the order of clever scientists. It portrays the path to knowledge as a difficult one, more often crossed by disappointments than by the discoveries that come as the final reward for long-sustained devotion to science and the improvement of human health. □

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Lemons and limes

Joe Collier

Drug Discovery: The Evolution of Modern Medicines. By Walter Sneader. Wiley: 1985. Pp.392. Pbk £12.95, \$21.95.

PHARMACOLOGISTS, physicians and legislators alike are now very wary of assuming that a drug is necessarily a medicine. Whereas a drug is any chemical substance that modifies a biological process, a medicine is that rare drug that can be used in patients to treat disease.

It is this distinction that occupies regulatory authorities (such as the Committee on Safety of Medicines, and the Food and Drug Administration) when licensing drugs for medical use; equally, it is the gap that the pharmaceutical industry strives to bridge so that new products can be marketed. Accordingly, as advances in fields such as biochemistry, genetics, immunology and pharmacology have led to the production of evermore sophisticated drugs, legislators have demanded increasingly detailed information about them before they will consider granting a licence. This balance now dominates the development of new therapeutic agents, and it is to this end that industry labours to discover how new drugs are absorbed, metabolized and excreted, how they should be formulated to be delivered to best advantage, how the dosage frequency should be adjusted to offer the greatest chance of efficacy, and, in longer term trials, the details of safety and efficacy.

Walter Sneader seems to be almost oblivious of these issues which are so central to the title of his book. They neither seem to be a consideration from which he might view the past, nor have they proved worthy of mention as a topic in their own right (and one so admirably dealt with by R.D. Mann in *Modern Drug Use: An Enquiry on Historical Principles*, published last year by MTP Press).

Instead, Sneader has filled the book with classical information. Each chapter (there are 16 in all) is devoted to a single broad therapeutic category, such as antibiotics, cardiovascular drugs, psychopharmacological agents, and in each the group is traced from the earliest reference to a seminal substance (penicillin, digoxin, Rauwolfia) through to drugs developed in recent history (the date seems to depend on Sneader's interests; inotropic agents stop with digoxin in 1933). The material is mostly in story form, in which the full names and addresses of each and every worker are listed, as are the movements of compounds and ideas between departments, companies and continents. There is an abundance of anecdotal information, clearly written and easy to follow — it is fascinating, for instance, to learn that scurvy returned as a threat to sailors when the trusty lemon juice was replaced by lime; lime juice, which was introduced for reasons of economy, has, we are told, about one-quarter of the ascorbic activity of lemon juice.

Two good reasons for publishing a new history book are that either it reveals fresh information or it analyses history from a



Eighteenth-century manufactory of drugs, with a direct sales outlet to the public (far right). The illustration appears in *The Development of a Medicine*, by R.B. Smith, recently published by Macmillan, London (hbk £30; pbk £12.95)/Stockton, New York (hbk \$60).

● Without fuss or pretension Smith's short book (160 pages) describes the development of medicines from their discovery (by serendipity or search), through their manufacture, their assessment both in the laboratory and the clinic, and on to the procedures involved in their marketing as licensed products. It gives a simple and straightforward account of contemporary practice in Britain, written by someone obviously familiar with the industry — when discussing drug company activity the book gives some rare glimpses of commercial philosophy in the pharmaceutical business. The book suffers from apparently being compiled in a hurry (tables illegible, legends wanting) but these should be corrected for the second edition that will surely come.

Joe Collier

novel point of view. Unfortunately, *Drug Discovery* does neither. The author's statements are always left open to question because no references are given in the text, so leaving the reader to search the rather poor and imprecise chapter bibliography at the end of the book. Interestingly, this sacrifice was made so that the text, which was written to convey "drama and excitement", should not be disrupted. However without references to contemporary information, the book has lost much scientific credibility.

Perhaps more annoying, there seems to be no benefit of hindsight. The ethical issues that have emerged so forcefully over the past 30 years and now engulf medicine seem to have left no impression. For its easy pace and the stories it relates I would expect the book to have its devotees; but as a serious historical account it falls short of what should be expected. □

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A stir in the primeval soup

Jacques Ninio

Seven Clues to the Origin of Life: A Scientific Detective Story. By A.G. Cairns-Smith. Cambridge University Press:1985. Pp.123. £9.95, \$17.95.

WE ALL admire the high technology feats that the simplest cells achieve in duplicating chromosomes, translating genes into proteins and adjusting their enzyme activities to their immediate needs. All such pathways are interdependent, each relying on all the others, as in an arch that would collapse if any one of its stones were missing. How did the first cells arise, if life requires, at a minimum, a translation apparatus to make proteins to replicate DNA?

The usual answer is that some 4×10^9 yr ago the Earth was a gigantic organic reactor producing masses of small molecules, amongst which were amino acids, sugars and nucleotides. Spontaneous polymerizations occurred, leading to nucleic acids that could replicate without the help of enzymes. Some of these "naked genes" acquired control over their environment. The coupling between nucleic acid replication and peptide synthesis became tighter and tighter until it became a strict coding relationship.

Cairns-Smith objects to this view; he believes that primitive organic reactors were more likely to produce tars and sludges than fine biochemical products, and that nucleotides are complex molecules that require sophisticated mechanisms for their production. Also, if a low-technology machine is to work, it must avoid the mutual dependency of its parts that is characteristic of more complicated machines. But, he says, the very existence of a primitive life-form made the synthesis of well-defined organic molecules possible. Amongst these, fancy polysaccharides, with no genetic use, might initially have been precursors of DNA or RNA. According to Cairns-Smith, new genes and the machineries they controlled gradually took over from the older forms of life: "None of the fibres in a rope has to stretch from one end to the other . . . new 'gene fibres' may be added and others subtracted without breaking the overall continuity".

These ideas are well known among scientists in general but are rarely discussed openly. In the origins-of-life research community, people seek a comfortable corner where they are least likely to interact with competitors. Yet, consider the fundamental issue: is nucleic acid replication conceivable in a prebiotic world? We are flooded with so-called theoretical treatments which generate models of the evolution of prebiotic self-replicating nuc-

Marking the path

Peter Laing

The Biotechnology Business: A Strategic Analysis. By Peter Daly. Frances Pinter, London/Rowman & Allanheld, Totowa, New Jersey:1985. Pp.155. £16.50, \$25.

THE emergence of an international industry based on molecular biology and immunology is a striking example of the potential economic benefits of government funding of basic research. Equally, however, the commercialization of biotechnology well-illustrates the familiar pattern of technological innovation being avidly and rapidly exploited in the United States and only belatedly in Europe.

In the United States, the availability of venture capital and an entrepreneurial business climate have led to the formation of numerous specialist biotechnology companies. These have been able to attract many of the brightest academic scientists (who would not normally have considered an industrial career) and concentrate upon the problems of developing marketable products. The large, established American companies, aware of the many studies that have shown that technical innovation most often stems from small, flexible and highly-specialized firms, have generally encouraged this process by the provision of research contracts (and even equity finance) for start-up companies, obtaining in exchange the commercial rights to future products.

By contrast, in Europe the start-up company as an engine of technological change has until very recently been a rarity. The brunt of commercialization of new technology has been borne largely by the established industrial companies, in which new ideas have a depressing tendency to be stifled or restrained by corporate inertia and the "Not Invented Here" syndrome. This situation has been compounded by a general lack of contact between industry and academia, particularly in the biological sciences, with the result that the more enterprising scientists have either remained in universities or left for North America.

In Japan, a strong sense of national purpose and a cultural preference for con-

sensus solutions have engendered active cooperation between Government, industry and academia with the aim of establishing national priorities for the exploitation of new ideas and identifying the means of bringing them about. Even so, the vehicle for commercialization in Japan has invariably been the established company — indeed, companies in moribund or declining industries have often been selected for revitalization by the injection of funds for biotechnology projects.

The Biotechnology Business is a comprehensive and readable guidebook to the commercial side of the biotechnology industry. As acknowledged by the author, it leans heavily on the excellent Office of Technology Assessment report *Commercial Biotechnology*, published in 1984, but is less nationalistic in its approach (and also weighs only a quarter of the OTA report's daunting 1.2kg).

Daly's emphasis on strategic analysis, made explicit in his sub-title, has resulted in many interesting case studies. Among them is a timely comment on the American company Genex, warning of the dangers of the company's heavy reliance on a single manufacturing contract for L-phenylalanine with G.D. Searle. This contract was terminated a few weeks after the book was published, plunging Genex into a financial crisis.

The source material for the book appears to have been culled largely from articles in specialist biotechnology magazines. These have tended to concentrate on the newsworthy start-up companies, and there is in consequence an under-representation of the strategies which are being adopted by the large chemical and pharmaceutical firms; now that the basic technology is more widely disseminated, such companies are beginning to make real inroads into the market. The likely impact of these moves on the start-up companies suggests not only a volatile employment market for the scientists involved, but, as Peter Daly points out, the development of an even more fruitful area for strategic analysis than the biotechnology industry has provided to date. □

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leic acid populations as though the very existence of such reactions were not the primary theoretical issue. With highly-skilled organic syntheses, the beginnings of something like non-enzymatic DNA or RNA replication are observed. But now, as Schwartz and Orgel have said (*Science* 228, 585-587; 1985), we need to "design reactions that proceed efficiently with more plausibly prebiotic substrate mixtures". Sooner or later, organic polymers, able to replicate in the laboratory and having simpler backbones than DNA or RNA, will be synthesized. Then the idea of "takeover", as Cairns-Smith has called it, will become inevitable.

Cairns-Smith, however, is far more radical than this, in that he rejects the concept of any primitive *organic* gene. Instead, he thinks in terms of clay particles, made of successive layers, each new layer added on top reproducing the defect structure of the previous layer; or of clays made of a succession of various kinds of uniform layers, that grow sideways. If such particles break across the layers, then the one-dimensional information contained in the particular succession of layers is conserved. Ways in which clays might influence organic reactions and use them for their growth are discussed in this book. Thus clay particles are suitable substrates for natural selection. Will field studies be conducted on the population structure of clay particles in given ecological niches? Or will some courageous young scientist design a clay-chemostat where the growth of clay crystals will be studied under various selective pressures?

Cairns-Smith's ideas deserve to be

assimilated and discussed. In *Seven Clues to the Origins of Life*, a layman's version of his earlier book *Genetic Takeover*, all his essential theses are presented in a clear, precise and condensed manner. The choice of a deductive form of presentation for his arguments (clays are introduced only three chapters from the end), buttressed by references to Sherlock Holmes, lends much rigour to the plot, and leaves no escape exit. How can low-technology life be designed, and how can it evolve towards the high-technology form? I know of no other book that succeeds as well as this one in maintaining this central question in focus throughout. It is a summary of the best evolutionary thinking as applied to the origins of life in which the important issues are addressed pertinently, economically and with a happy recourse to creative analogies.

The concept of "takeover" is an important evolutionary one, and may apply to a wide variety of situations. I regret in this respect that the author did not attempt to analyse concrete biological examples (such as the origin of split genes, RNA catalysts and ribosomes, or the design of the immune system) in the light of this idea. Inevitably, I think, schemes in which things acquire complexity of detail without a change in their organization will have to compete with "takeover" schemes. The time has now come for those scientists working on the subject of the origin of life to cease dealing with such ideas obliquely. □

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that may occur in any population of individuals". He transferred this anti-essentialism from entomology to human behaviour. Human sexual behaviour cannot be pigeon-holed; just as the world of wasps was a continuum in each and every one of its aspects, so too the male population cannot be divided into two discrete groups, heterosexual and homosexual.



The enigmatic smile of a swan — or an upside-down flamingo?

Gould teases out the wider implications of a particular scientific enquiry, from the taxonomy of wasps to a more accurate — and tolerant — understanding of what is regarded as sexual deviation. The scientific, moral and political (Kinsey had the misfortune to coincide with Senator McCarthy) implications are shown to interact.

The essays range widely from speculation about the reason for the extinction of the dinosaurs to the bizarre and unhappy cases of the Hottentot Venus and of Ritta-Christina, the celebrated siamese twin sisters. The style is relaxed but not flip; the content all the better for being disciplined by the limits of the monthly column. If there is a weakness it is Gould's rather too obvious wish to hook his reader with a startling opening paragraph. An essay on continuity and quirkiness in evolution leads off with Michelangelo and one on the flamingo's eating habits with Buffalo Bill. It is good journalism but unnecessary: in all these essays the subject matter is compelling enough in itself.

Scientists who communicate their enthusiasm to a wider audience are regarded by some of their professional colleagues as vulgar; if it can be explained to a layman it cannot be serious. Gould brushes aside that mixture of arrogance and insecurity. His essays popularize science in the same sense that Macaulay popularized history — by making it interesting and entertaining to read. Roll on volume five. Meanwhile I shall be looking for copies of volumes one to three. □

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Appeal to the laity

John Rae

The Flamingo's Smile: Reflections in Natural History. By Stephen Jay Gould. W. W. Norton: 1985. Pp. 476. \$17.95. To be published in Britain in February 1986, £12.95.

It is 20 years since C. P. Snow described the gulf fixed between scientists and non-scientists in academia. Not much has changed. The two communities continue to go about their business like a couple of Englishmen travelling in a foreign land: they are on the same journey but have nothing to say to one another because they have never been introduced. The non-scientist complains that the scientist cannot communicate what he is doing in a language that the layman can understand. The scientist complains that the non-scientist defines language in narrowly non-scientific terms — why should a biologist have to explain what he means by morphology any more than a grammarian has to explain what he means by tautology? No doubt it is the education systems

that are to blame; meanwhile the dialogue of the deaf continues.

Stephen Jay Gould overcomes this problem, not by laicizing the language, but by using it in the context of intellectual enquiries that any intelligent person can understand and by a technique of cross-referencing that relates the scientific enquiry to comparable problems in other disciplines. It might be argued that as a palaeontologist and evolutionary biologist he has an easier task than say, the nuclear physicist. But easy or not he succeeds brilliantly. This collection of essays, the fourth to be based on his monthly column in the *Natural History Magazine*, communicates both the fascination and the universality of scientific study in a way that can hardly fail to intrigue scientist and non-scientist alike.

Take, for example, Kinsey and the wasps. Kinsey's reports on sexual behaviour were not the product of his desire to promote sexual enlightenment. He drifted into sex research by accident and applied to the question his experience as America's leading taxonomist of wasps. His study of wasps persuaded him — in his own words — "of the uniqueness of individuals and of the wide range of variation

Biology and the behaviour of man

John Maynard Smith

Vaulting Ambition: Sociobiology and the Quest for Human Nature.

By Philip Kitcher.

MIT Press: 1985. Pp. 447. \$24.95, £24.95.

Do we really need another critique of sociobiology? In general, probably not, but perhaps we need this one. Kitcher, like everyone else, approaches the problem with prejudices, but he tries harder and more successfully than most to rise above them. Prejudices are inevitable. It is natural for geneticists and evolutionary biologists to hope that their disciplines will throw new light on the human condition, and equally natural for social scientists to resist the threatened takeover. More important for many of us, previous efforts to apply biology to human affairs have too often ended up as justifications for racial, sexual and class inequalities. Kitcher, who grew up in England, has not forgotten that, in the post-War years, schoolchildren were divided at the age of eleven into sheep and goats, and that this division was justified by the leading experimental psychologists of the day. He and I share this experience — he as a tested child and I as a parent of tested children. It has left us cautious about proposals to use biological theory to plan human institutions.

Kitcher, then, is unsympathetic to the claim that evolutionary biology can guide

... what we now need in sociobiology is a more cautious analysis of the data ...

political judgement, and I suspect he was unsympathetic before he started work on this book. Unlike some other authors, however, he has undertaken a genuine study. He does understand the ideas he is criticizing. He has the biological knowledge to evaluate the evolutionary background to sociobiology, and the mathematical ability to analyse the claims made for it. Above all, he presents sociobiology in its strongest and most coherent form, and avoids the easy option of attacking only its more idiotic manifestations.

He distinguishes sharply between the attempt to understand the evolution of social behaviour in animals, and attempts to understand man. He is sympathetic to the former enterprise. Correctly, he points out that there is no special underlying theory: "There is no autonomous theory of the evolution of behaviour. There is only the general theory of evolution". It may be that interactions between relatives, and frequency-dependent fitnesses, were more important in the evolution of the behaviour of birds than in the evolution of their wings, but they are not peculiar to behavioural evolution: kin selection and game theory are just as relevant to plant evolution.

There is, of course, good and bad work in animal sociobiology, and Kitcher gives examples of both. The bad, he points out, has two characteristics: data are quoted as supporting some specific hypothesis, without considering alternatives, and the hypotheses themselves are modified after the fact until data and predictions are brought into line. However, his chapter "Dr Pangloss's Last Hurrah", which takes issue with the "adaptationist program", seems to me only partly correct. He presents two genetical reasons for not expecting perfect adaptation. The first is that there are genetic systems, even with constant fitnesses, in which selection will not fix the fittest genotype. The simplest is that of heterozygous advantage: if Aa is fitter than AA or aa, selection cannot produce a population consisting entirely of Aa individuals. This is of course true, but is it interesting? If we want to understand why some species does not have the phenotype predicted by theory, this kind of genetic detail is rather unlikely to be the reason. Suppose, for example, we are interested in the shape of vertebrate wings. Aerodynamic theory shows that the optimal shape is usually elliptical. Pterodactyls, however, never had elliptical wings, but no one would explain this by suggesting that, perhaps, only heterozygotes had elliptical wings. The true explanation has to do with the way in which pterodactyl wings were made. Of course, this would be reflected in the absence of certain kinds of heritable variability, but that is not a useful way of thinking about the problem. For phenotypes of the complexity typically discussed by sociobiologists, it is usually better to think at the level of development and physiology than of genetics.

All the same, there will be cases in which this kind of genetic constraint will be relevant. The second kind of genetic constraint discussed by Kitcher, however, seems to me to be a misunderstanding. He points out that when fitnesses are frequency-dependent, the mean fitness of a population may decrease under selection. Therefore, he says, "proponents of optimization analyses who show that a certain design would maximize mean fitness may not automatically assume that selection can produce this design". Now, as Kitcher understands very well, a major thrust of sociobiology has been to show that selection acting at the level of the individual does not necessarily lead to the evolution of characteristics optimal for the population. One of the main reasons for

this is that fitnesses are frequency-dependent. Evolutionary game theory was developed specifically to analyse such cases. It is not sociobiologists who suppose that selection "maximizes [population] mean fitness". It is ironic that the phrase "Pangloss's theorem" was first used in the debate about evolution (in print, I think, by myself, but borrowed from a remark of Haldane's), not as a criticism of adaptive explanations, but specifically as a criticism of "group-selectionist", mean-fitness-maximizing arguments.

Thus I think that Kitcher is unfair to sociobiologists when he introduces the argument from frequency-dependence. However, it is a rare slip: in general his

... It is natural for geneticists and evolutionary biologists to hope that their disciplines will throw new light on the human condition ...

account is just. I agree that what we now need in sociobiology is a more cautious analysis of data, and a more careful consideration of alternative hypotheses. This will not come easily, for reasons that are as much sociological as scientific. The critique by Gould and Lewontin has had little impact on practitioners, perhaps because they were seen as hostile to the whole enterprise, and not merely to careless practise of it. Theorists like myself are understandably delighted when some set of observations seems to fit their theories. Field workers, equally understandably, are pleased if their data receive a rational explanation. The time has come, however, for editors and referees to place more emphasis on the quality of the data, and the care with which alternative explanations have been considered, and less on success in fitting the data to some particular theory. But, as Kitcher insists, the enterprise is worthwhile, and the best work is of a high standard.

What of man? Clearly, biology must have something to say. Man is an animal, and has evolved by the same processes as other animals. The debate is between those who, while accepting that man is an animal, argue that he is such a peculiar animal that evolutionary biology can have little to say about his social behaviour, and those who think that the study of human societies, just as of ant societies, must be rooted in biology. The second position Kitcher refers to as "pop sociobiology". I think this is a pity, for two reasons. First, it gives an image of superficiality and appeal to popular prejudice which, at least sometimes, is quite unfair: it is hard to imagine anyone less "pop" than Richard Alexander. Second, it gives the wrong impression of what Kitcher himself is doing: he is scrupulous about putting the best interpretation on sociobiological arguments. But I see his difficulty: we do need a term for the application of sociobiology to human beings, and I have no better one to offer.

Kitcher's basic position is that one cannot dismiss pop sociobiology simply by asserting that it assumes genetic determinism and is therefore false, since plausible sociobiological arguments can be developed which do not assume that genes determine behaviour. There is therefore no escape from considering these arguments in detail, and to see if they stand up

... one cannot dismiss pop sociobiology simply by asserting that it assumes genetic determination and is therefore false ...

and deliver any fruit. Kitcher distinguishes two schools of pop sociobiology, those of E. O. Wilson and of Alexander. Wilson's basic argument he sees in the form of a ladder, as follows:

(i) We can plausibly argue that the members of some population, G, would maximize their fitness by exhibiting behaviour B.

(ii) If we observe that members of G in fact do B, we conclude that B became, and remains, prevalent through natural selection.

(iii) Because selection is effective only if there are genetic differences, we can conclude that there are genetic differences between current members of G and their ancestors, who did not do B.

(iv) Because there are genetic differences, and because the behaviour is adaptive, the behaviour will be difficult to modify by altering the social environment.

This is a shortened version of Kitcher's reconstruction. A major part of his book consists of a step-by-step critique. Clearly, the last step is based upon the shakiest grounds: the fact that our ancestors did B in all previous environments is not proof that they will do B in a wholly new one. The first three steps look more secure. Kitcher's most effective criticism here is not of the logical possibility of taking these steps, but of the ways in which they are in fact taken. For example, consider sexual behaviour. He quotes Wilson as espousing the view that evolution will lead to males that are "aggressive, hasty, fickle, and indiscriminating", and females that are "coy". But theory suggests not one evolutionary optimum, but several, and a number of our primate relatives form long-term pair bonds and show extensive male parental care. Hence there is little justification for Wilson's first step onto the ladder.

A second illegitimate way of getting on to the ladder is to apply to animals words which describe some human behaviour. For example, mallard drakes are said to "rape" ducks. Now it is true that drakes do force copulations on ducks and, by so doing, probably increase their fitness. What is the harm in calling this rape? If you are interested in ducks, rather little, but if you are interested in people, quite a lot. It implies that human rape occurs because it increases the inclusive fitness of the rapist. The contexts in which rape

occurs makes this implausible. I agree that there is a danger in applying words such as "aggression", "incest", "homosexuality" and so on to animals and man alike, when the behaviours referred to may be quite different. However I do have reservations. The alternative is often to invent a turgid and incomprehensible vocabulary to describe what animals do. I remember our unsuccessful attempt to introduce the term "kleptogamy" at an ethological congress, because we feared that the Anglo-Saxon alternative might offend our hosts.

A few years ago, I worked through the equations in Lumsden and Wilson's *Genes, Mind, and Culture* and found them to be badly flawed. Kitcher has done a still more thorough job, and come to essentially the same conclusion: his chapter is entitled "The Emperor's New Equations". On three occasions Wilson has found it helpful to find a mathematical collaborator. His first two were Robert MacArthur and George Oster: he was third time unlucky.

The second approach to human sociobiology, taken, for example, by Alexander, Irons, Chagnon and Dickemann, is more direct. Man is treated like any other animal. The question asked is as follows: given the social environment, do people behave so as to maximize their inclusive fitness? The answer, it is claimed, is "yes". Unlike Wilson's arguments, which seem to me generally ill-formulated and empty of content, this claim is worth taking seriously, even though it is probably false. Kitcher, attacks it on two fronts. First, he asks what proximate mechanism could possibly bring such behaviour about, since it seems to require an unconscious relationship-calculator and fitness-maximizer influencing our conscious actions. If I were Alexander, I would reply that, if the claim is true, then it is up to psychologists to discover the mechanism.

Kitcher's second line of attack is to ask whether people do in fact maximize their fitness. Here, the test case is Dickemann's account of societies practising female infanticide, and other acts not obviously contributing to fitness. Kitcher gives a careful analysis of this case, and develops a mathematical model of it suggesting that the increases in inclusive fitness that Dickemann proposes would not in fact occur. I am not sure whether he is right, but this is where the action is. This school of sociobiologists do say things about real societies that are testable; I find it hard to believe that they are right, but at least they are not vacuous.

This is an admirable book. Kitcher has the necessary background in biology, mathematics and philosophy. He is aware of his prejudices, and does his best to overcome them. This will not be the last word, but it is the best one yet. □

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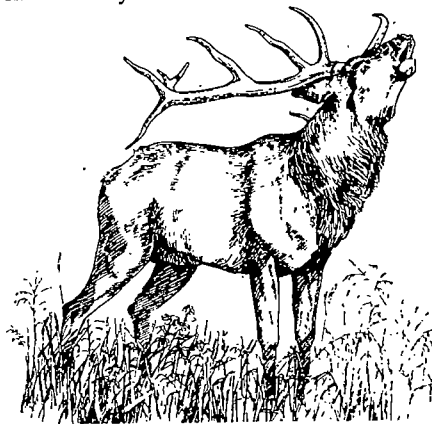
A case of peripatetic mammaldom

David W. Macdonald

Naturalized Mammals of the World. By Christopher Lever. Longman: 1985. Pp. 487. £40. To be published in the United States late 1985/early 1986, \$79.95.

THE innocent naturalist might relish a glimpse of an elusive mammal as a glimpse of unspoilt nature — a sight that might have brought a thrill in just that place, under just those circumstances, for millennia. Surely mammals, of all creatures, with their unassailable wildness, and secretive and often nocturnal habits, remain untainted by the meddling hand of man, albeit restricted to dwindling enclaves of suitable habitat?

Naturalized Mammals of the World shatters any such illusion that wild mam-



Red deer — now a New World resident.

mals are where they ought to be, or rather where they used to be. The reader will not be many pages into this intriguing catalogue of mishaps before succumbing to the overwhelming impression of a peripatetic mammaldom, of which an astounding array of displaced species are wreaking biogeographical havoc. Most people could name a handful of aliens in their country — in Britain, for example, the grey squirrel and coypu — but common knowledge of the few belies the many less-notorious species which have been deliberately (and, generally, in retrospect, misguidedly) shuffled around the globe. I got up to 67 major introductions before losing count. To qualify for the doubtful distinction of inclusion in the book, a species should have been imported from its natural range to a new country or region either deliberately or accidentally by human agency, and should currently be established in the wild in self-maintaining and self-perpetuating populations unsupported by, and independent of, man.

Apart from a succinct introductory section, and a set of useful tables and appendices, the book is made up of single-species monographs, each of which documents that species' displacement and sub-

sequent misdeeds. An addictive feature of these monographs is the pair of contrasting world maps provided, showing the animal's range "before" and "after" the onset of globe-trotting; the temptation, which I suspect few will resist, is to thumb ahead for a furtive peep to discover whether your favourite species suffers the stigma of far-flung black blobs scattered improperly across the "after" maps. The text itself is an enjoyable blend of biology and history, and is written in a precise and scholarly style. Although the author does not dwell on the implications for ecological theory of the effects of different immigrants on their new habitats, his text draws attention to the extraordinary interest of the process of invasion, and the many "unnatural" experiments that have been inadvertently established worldwide.

The history of three canids illustrates the scope of the book and the variation in the examples it documents. Red foxes were introduced to Australia in the late nineteenth century, in numbers generally no greater than two's and three's, by would-be hunters. Their progress can be charted in detail year by year from local records, until they are now widespread to

the extent that tens of thousands of descendants of the original few are killed annually. (Incidentally, British foxes were also shipped across to populate the eastern United States, and foxes from Spain were imported to bolster the numbers in Britain.) Then there is the raccoon dog, which originated in the Far East. For them, the scale of the operation was quite different. Between 1929 and 1967, 8,651 were released in European Russia by those with an eye on their pelts — in 1947 alone, 1,147 gravid females were let loose in the Caucasus. Each of these cases differs from that of the dingo, which was probably shipped to Australia some 3,000 years ago and so must soon qualify for honorary citizenship.

Although essentially a reference volume, this book has the "Guinness Book of Records" type of appeal to a browser. Apart from serving its main purpose, it will doubtless provide an abundance of worthwhile "Did you know . . . ?" gambits for both amateur and professional naturalists. □

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Botanical spread

Brian Mathew

A Guide to the Vegetation of Britain and Europe. By Oleg Polunin and Martin Walters. Oxford University Press: 1985. Pp.284. £17.50, \$25.

AMID the dense rash of wild-flower books dealing with the flora of Britain and Europe at the species level, it is refreshing to be presented with a volume devoted to whole communities of plants. Polunin and Walters are probably accurate in claiming that this is the first attempt to bring together all the information on the subject at a layman's level, although, as they acknowledge, there are many other detailed works covering individual countries or regions.

Accordingly, a helpful bibliography has been included, divided into vegetation types, for those who wish to pursue any interesting points raised in the text. For internal reference, the reader is also well provided for, with indexes to Latin names, English names and plant communities, and a useful glossary (although I was surprised to find no mention, here or in the index, to the commonly used term "downland"). Conservationists, who now tend to put as much emphasis upon the preserva-

tion of habitats as of individual species, will find much useful material is provided, and there is a descriptive list of the Nature Reserves and National Parks throughout Europe.

The opening chapters deal with the all-important subjects of soils and climates, which have a great bearing on the plant cover, and there is an interesting summary of the history of Europe's vegetation from the earliest fossil records onwards. The bulk of the book consists of 14 chapters devoted to different regional zones — Arctic, Mediterranean, Central Europe and so on — and, within these, there are subdivisions down to communities with examples of the species to be found in them. The text is supported by many good line drawings and a few rather poorly reproduced monochrome photographs; the latter, however, are more than compensated for by the 110 excellent colour plates of vegetation types.

The authors have faced a difficult task, and it is not surprising that the book may appear bitty at first sight. As with most aspects of the botanical world, the demarcation lines are often ill-defined and there is much overlap, for example between *maquis* and *garigue*, with several different types of each of these. To have presented an over-classified statement of the situation would have led to many inaccuracies, and it is to the particular credit of Walters and Polunin, given the intended audience of their book, that they have struck the right balance between the superficial and the overlearned. □

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Animals and plants first

Michael J. Bean

International Wildlife Law. By Simon Lyster. *Grotius, Llandyssul, Dyfed SA44 4BQ, UK: 1985. Pp.470. Hbk £25, \$37; pbk £12, \$17.50.*

THE earliest efforts at international cooperation in the conservation of living resources date back well into the nineteenth century. Since then, the number of international treaties and agreements aimed at conserving wildlife has grown steadily. Not until Simon Lyster's book, however, has anyone tried to offer a comprehensive analysis of that body of law. The result is a compelling display of legal scholarship that should reward scientists interested in policy matters and conservationists around the world.

This is a book by a British lawyer about international conservation law. It escapes, however, the trap of appealing only to a narrow audience of similar specialists. After an introductory chapter that provides a layman's overview of certain general principles and characteristics of international law, Lyster turns his attention to a series of wildlife treaties. For each of these he offers some historical background, including a discussion of the problems that the treaty was intended to address, a detailed analysis of the terms of the treaty, and an assessment of the treaty's actual implementation and effects.

The most remarkable aspect of the book is its sheer scope. It is not limited to the treaties of a particular country or geographical region, and reaches out across ocean barriers to encompass the Convention on Nature Protection and Wildlife Preservation in the Western Hemisphere (1940), the African Convention on the Conservation of Nature and Natural Resources (1968), a pact of 1969 among Andean nations for the conservation of the vicuna, an agreement of 1973 among circumpolar nations for the conservation of the polar bear, numerous European treaties for the conservation of birds and other wildlife, and yet other initiatives.

The most thorough analysis in the book is devoted to four treaties of the 1970s that Lyster calls "the centrepiece of international wildlife law": the Convention on Wetlands of International Importance Especially as Waterfowl Habitat (1971); the Convention Concerning the Protection of the World Cultural and Natural Heritage (1972); the Convention on International Trade in Endangered Species of Wild Fauna and Flora (1973); and the Convention on the Conservation of Migratory Species of Wild Animals (1979). The complete texts of these and eight other treaties are reprinted in full in

● *Flowering Plants of the World*, consultant editor V. H. Heywood, which was published in 1978 by Oxford University Press and has been out of print, has been re-issued by Croom Helm in Britain and Prentice-Hall in the United States. Price is £19.95, \$39.95.

an appendix that occupies nearly a third of the entire volume.

The criteria upon which Lyster bases his judgement that the four treaties he principally addresses are in fact the most important are unstated. All are indeed global, open for signature by any nation of the world, but only two, the World Heritage Convention and CITES (the Endangered Species Convention), have the adherence of a majority of the world's nations. The Migratory Species Convention may be the most recent of international conservation initiatives, but with only 15 party states and no real accomplishments to date, Lyster's claim that it occupies part of the "centrepiece of international wildlife law" is premature at best. The Wetlands Convention, though it has served to direct attention to a particularly pressing problem; is notable mostly for its failure to create clear, enforceable obligations. While it is dealt with elsewhere in the book, the International Convention for the Regulation of Whaling might more properly have been included in the centrepiece if world attention and actual results were the criteria for that uncertain distinction.

A recurring theme throughout the book is the wide, sometimes enormous, gap that separates the aims and apparently literal meaning of most of these treaties and their actual implementation. For example, the World Heritage Convention tries to come to grips with the reality that many of the world's great cultural and natural resources are in the countries least able financially to assure their protection or forego the short-term benefits associated with their exploitation. The mechanism fashioned to answer this dilemma is, through the creation of a trust fund, to transfer wealth from the developed to the developing world for purposes of conservation. Though there is nearly universal agreement that such a transfer is needed if the intentions of the Convention are to be realized, the actual amounts of money made available for the purpose have been so small as to undermine any confidence that the commitment to the principle is anything more than cosmetic. Beyond noting the fact of such gaps between promise and performance, Lyster does not delve into explanations or suggest possible solutions.

His book stands, however, as an impressive survey of some of the efforts at international cooperation made over the past century to stem the loss of wildlife. It is, at the same time, a depressing reminder of how short many of those efforts have fallen. □

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• Reviews of *Mind and the New Physics* by Fred Alan Wolf, *the Dialectical Biologist* by Richard Levins and Richard Lewontin, and *The Background of Ecology* by Robert P. McIntosh will appear in future issues of *Nature*.

More Darwinian detractors

Mark Ridley

Evolution: A Theory in Crisis. By Michael Denton. Burnett, London: 1985. Pp.368. £17.50. To be published in the United States in April 1986 by Adler & Adler, Washington, DC.

Creation and Evolution: The Facts and Fallacies. By Alan Hayward. Triangle, London: 1985. Pp.232. Pbk £2.75.

Adam and Evolution. By Michael Pitman. Rider, London: 1985. Pp.268. £9.95.

Evolutionary Theory: The Unfinished Synthesis. By Robert G.B. Reid. Croom Helm/Cornell University Press: 1985. Pp.405. £25, \$34.50.

WRITING a review of some long since forgotten anti-Darwinian work in this journal 95 years ago, Raphael Meldola remarked that "it is hardly necessary to say that many of the most weighty objections have been culled from the writings of Darwin himself". *Toutes ces la même chose*. Another four books published during 1985 now confirm that Mr Darwin's critics are as active, and their procedures the same, as ever. I do not think it is worth replying to them here. Readers of *Nature* can scarcely need to be put right on these old issues; and, anyhow, Darwin himself and many others have dealt with them at the length they deserve. How can I hope to succeed with three authors (Denton, Hayward and Pitman) who, like the Victorian astronomer Sir John Herschel, think that evolution by natural selection is the "law of higgledy-piggledy" — a "random search mechanism" (Denton), of "pure chance" (Hayward and Pitman)? Or with another author (Reid) who, like the Duke of Argyll, thinks that there must be some grave defect in Darwin's theory, because it personifies Nature in its analogy with artificial selection?

Denton, Hayward, Pitman and Reid, then, are opposed to the Darwinian theory. The first three of them (as we shall see) object to it for similar reasons; but they differ in what they would put in its place. Hayward is a creationist and Christian. Pitman is a creationist too, and probably a Christian one, although he has heretical leanings towards the dualistic (not trinitarian) wisdom of the East and an enthusiasm for music mysticism. Denton's purpose is purely destructive: he has no alternative to offer. Reid thinks he has a secular alternative, which we shall come to. His is the only book of the four that is written in a cerebral style, for an audience of professionals of some kind. Denton, Hayward and Pitman all write for uninformed readers, the latter two in the kind of short, didactic sentences and superficially patient tone that, in Britain, one associates with the closed minds of their

school. Denton has a different style. His pen is constantly running out of control. Every few pages he treats us to some more or less silly exaggeration. The preface sets the tone by telling us that "every aspect of evolution theory is being debated with intensity", at conferences, in journals and in the Natural History Museum in London. Denton may think that exaggeration is a necessary part of popular writing. If so, he is wrong.

None of the authors is an evolutionary biologist. Denton is a biochemist; Reid a philosophical physiologist; Hayward a retired physicist; Pitman "has an MA in classics". They would not think this a disadvantage. A Darwinian education (they believe) cripples the mind, filling it with emotional prejudices. Hayward accordingly prefers the unbiased view of the "outside observer", and Bernard Stonehouse without irony recommends Pitman to us as follows: "his unorthodox background must have helped Michael Pitman to write this book; it might not have occurred to one formed in a more conventional mould".

Their procedure is to sift through the writings of Darwin, and such popular secondary and tertiary sources as Stephen Gould's essays, *New Scientist* and even *The Guardian*. From this material, they seize upon the bits that look like difficulties for Darwinism, and ignore everything else. Then, after surrounding the difficulties with schoolroom rhetoric, sub-Kuhnian psychobabble and suitably simplified Victorian history, they send the whole to press. As argument, indeed, all four books are sad stuff. The reasoning I shall come to, but mark well the style. Denton is especially keen on the device (it must have a technical name) "even Darwin-Simpson-Sir Peter Medawar admits-concedes-acknowledges that . . .", which he places before each attenuated quotation, in order to reverse their meanings. I similarly found myself, in Hayward, "grudgingly admitting" something that in reality I unhesitatingly espouse. Denton's characterization of Lyell provides another example. "For most of his life", we are told — and this is all we are told — "[he] was vigorously opposed to the idea of evolution".

So why do Denton, Hayward and Pitman object to the neo-Darwinian theory? Their case (the arguments of the three are similar) is essentially Victorian. It plays on two main "difficulties": "chance" and "gaps". Natural selection, they all allow, can cause small changes in simple adaptations; but they deny that it can cause large changes among types and the evolution of complex adaptations like the vertebrate eye. "Different processes altogether may be involved, and here Darwin's strength fails" (Pitman). As they have identified natural selection with chance, they deduce (correctly) that it could not in principle produce large directed changes. Only the premise is wrong. In the Darwinian

theory, natural selection is not a chance process and complex adaptations evolve in many small changes. That possibility they either silently ignore, or misunderstand and quickly brush aside.

Then we have the problem of gaps. There are gaps between the classes of modern forms, and those well-publicized gaps in the fossil record. The origin of life is an example. According to these authors, it would not be possible for evolution by natural selection to cross the gap between the non-living and the living. After all, where are the intermediate forms? They have not been found, even though, according to Pitman, "billions of dollars are spent annually in attempts to demonstrate abiogenesis under laboratory conditions". Pitman also quotes Francis Crick, who "admits" that "regarding the origin of life we often find 'too much speculation running after too few facts'"; but speculative pursuit is as ill-advised when the aim is to demonstrate a negative, as a positive, conclusion. Darwin's own remarks about difficult transitional stages — that they are a problem of the imagination, and not of the reason — is silently ignored, but implicitly illustrated.

The theory of "punctuated equilibrium" of Eldredge and Gould here again does time for the creationist cause; but the Darwinian view (and that of Eldredge and Gould although they do not like to say so), that the gaps of the fossil record are due to its incompleteness, is scarcely discussed. The gradualism of Darwin, which applies not to rates of evolution but to the evolu-

tion of complex adaptation, is misunderstood by all the authors.

They have other objections too. For instance, they have all learned, from the popularizations of Sir Karl Popper and others, that natural selection is a circular argument (although Denton has still to learn from Pitman that Popper has "to some degree relaxed his position in a way that favours Darwinism"). And Denton thinks that natural selection cannot explain homology, including serial homology which "cannot by any stretch of the imagination be explained by descent from a common ancestor".

In the case of the two main objections I have dealt with, the authors have neglected the answers put forward by Darwin and his followers. Why should this be? Maybe they are unaware of them. Maybe they think only the devil needs an advocate. The texts, however, point to a third reason. The typical Darwinian of these pages is a man incapable of rational argument. Neo-Darwinism (we are told) is an "article of faith" (Pitman), a "belief-web" (Reid), a "paradigm" of "illusion" and "dogma" (Denton). It is believed in, and defended against critics, only because of "emotional commitment" and "extra-scientific factors", which are to be understood by the "sociology of knowledge". When the dogmas of neo-Darwinism are threatened, for instance by neo-Lamarckism, its fanatical adherents do not react with rational scepticism, about dubious factual claims. They remorselessly persecute the poor heretics, until they are driven into suicide or emigration, and

then trumpet their triumph in a ritual of collective orthodoxy. The doctrine of neo-Darwinism is thus seen as establishment cant, not rational science, and therefore hardly merits serious consideration. The critic may save himself the trouble, and ignore it.

Reid is a rather different kettle of fish. His purpose is not, for the most part, destructive. He does include a chapter on some of the "difficulties" in Darwinism, and he notices others from time to time. The main problem, he believes, is that Darwinism is "reductionist" and has therefore "shied away from explaining the emergence of meaningful wholes from individually meaningless parts". It also suffers from two previously unnoticed defects: a new kind of circularity, and the "homeostasis paradox" ("if homeostasis is characterized as constancy, and evolution characterized as change, how did the homeostatic condition evolve?"). The circular argument is this:

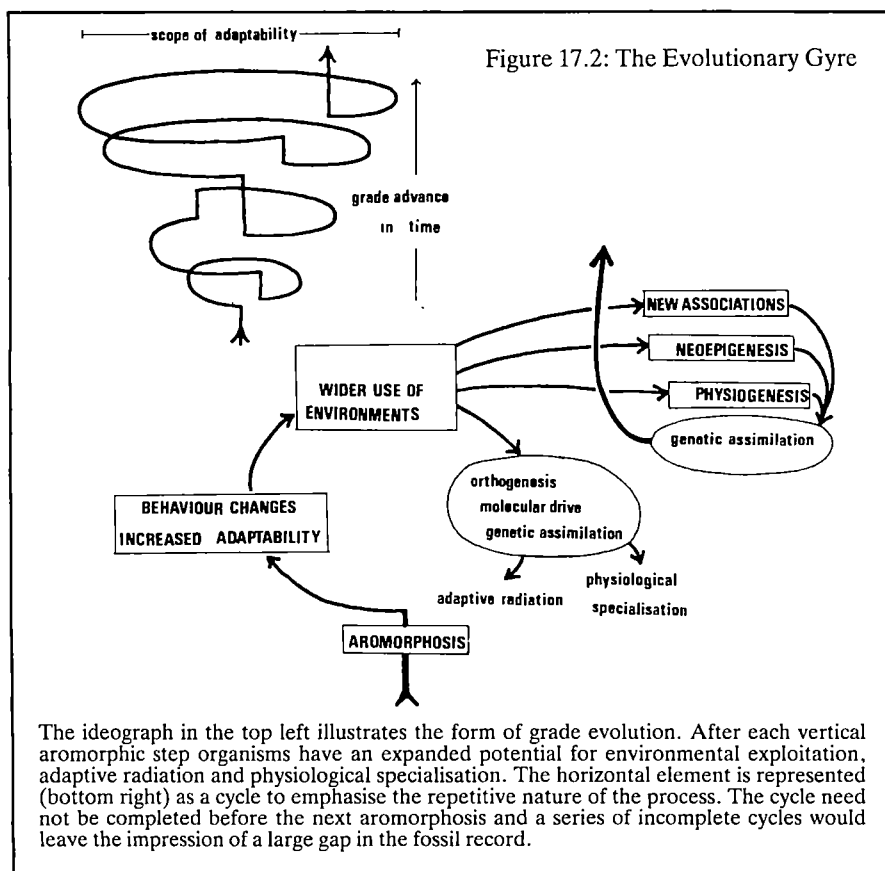
Descent by modification through natural selection was Darwin's slogan. This linkage of two independent ideas was the core of Darwin's thesis and claim to originality, although it involved the circular argument that evolution occurs because of natural selection, therefore natural selection must operate; natural selection operates, therefore evolution must result.

Reid does not name names here, which is a pity because I cannot think of anyone who has fallen into this error.

But Reid's main purpose, as I have said, is positive. He was woken from his Darwinian slumbers by the student rebellions of the late 1960s and a reading of Thomas Kuhn. He duly discovered an alternative paradigm, of holists, and it is his aim in his book to introduce us to them. He has arranged people such as Driesch, Woodger, Bergson and von Bertalanffy into convenient groups; for each he gives some biographical details and summarizes their attitudes. They are in favour of emergence, integrative hierarchies and all that sort of thing. Anyone who is looking for an introduction to these people may find the book useful. But no one else will, because it is neither historical nor argumentative; it does not put their opinions in context nor does it try to show us that they were right.

Reid ends by expounding his own holistic alternative. It defies short summary, but fortunately he illustrates it with some figures, the most important of which is reproduced (left). Reid does not like neo-Darwinians, who do not treat all his friends and their enthusiasms with sufficient gravity. But now we have an opportunity to set things right. We should add the "evolutionary gyre" to our introductory lectures, expose our students to the alternative paradigm, and treat it with the seriousness it deserves. □

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Holding back on dangerous play

Lawrence Freedman

Superpower Games: Applying Game Theory to Superpower Conflict. By Steven J. Brams. *Yale University Press: 1985.* Pp. 176. Hbk \$31.50, £22.50; pbk \$9.75, £6.95.

THERE was a time when game theory was all the rage in the strategic studies community. This was the "golden age" of strategic studies—the late 1950s and early 1960s—when it was fresh with new ideas and insights on how best to deal with the nuclear dilemmas that were perplexing policy-makers.

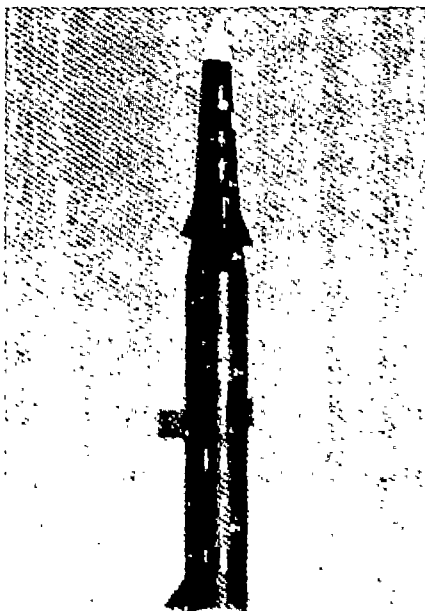
Game theory was ideal in two ways. First, it offered a methodology of some sophistication with which to challenge the traditional approaches to strategic issues based on history and politics and military experience. All of these were now deemed to be irrelevant in the nuclear age. Second, the assumptions behind game theory fitted in with the predispositions of the new generation of strategists. It was not that they saw superpower conflict as a "game"; the term only really referred to the idea of structured situations in which the ability of either "player" to achieve his best outcome depended on decisions taken by the other.

Superpower conflict at the time seemed to be suited to game theory for three reasons. First it involved two sides who were deeply antagonistic and likely to be so for the foreseeable future: there was no risk of the game dissolving in an outbreak of peace and harmony. Second, despite this, the antagonism was incomplete. There was some common ground—to take the most obvious, a mutual interest in the avoidance of an outbreak of nuclear war. Third, as on the one hand the two sides were engaged in a contest for power and influence in the world, while on the other a nuclear war in which both would be devastated was the most likely result of any attempt to resolve differences through military means, it was useful to have a tool for identifying the solutions that logic might lead both powers to adopt in particular types of situation. If some solutions pointed to disaster then corrective action would need to be applied.

Within its limits game theory was quite valuable. It served as a means of emphasizing the extent to which superpower confrontation did not necessarily mean that one gained what the other lost (zero sum-games) but that there could be circumstances in which both won or both lost (non-zero sum games). It drew attention to the extent to which in non-zero-sum games both sides might tend towards the best of the worst solutions (minimax). Following through the logic of certain games

could illuminate aspects of the real dilemmas that the superpowers faced. But the approach did have its limits: the individual games had to be simplified in order to keep them manageable and a presumption of rationality had to be made. Moreover, the results of the games depended critically on the assumptions made as to the choices available to the two sides, and the values attached to the alternative outcomes (payoffs).

Accordingly the best exponents of game theory (notably Tom Schelling) never allowed themselves to be restricted by the theory and were often at their most effective in using it only as a starting point for a broader analysis. Those who had dabbled with it when fashionable soon lost in-



Pershing II missile — large chip in a deadly game.

terest. Nevertheless a number of scholars have persisted with the development of the technique.

Steven Brams is one such scholar who has worked hard to extend the range and applications of the basic theory. This book, which is made up of previously published articles, updated and reworked to give them some coherence, contains the results of his work. He is an unqualified believer in its continuing relevance. In the concluding chapter he states: "The evidence seems to me, incontestable that game theory captures a substantial part of the logical basis and empirical reality of superpower conflict and provides a unified framework for its analysis" (p. 145).

This case can be assessed by examining the quality of the analysis and then the value of the conclusions. For the first, it has to be said that Brams makes few concessions to his reader. He does not attempt to justify his methodology against the standard objections or to introduce it for a lay reader. The mathematics is hard work for those (such as myself) who are

out of practice, and the argument is tight. So all that can be said, with the utmost humility, is that it seemed fine to me.

Much of the development of theory in this book constitutes an attempt to catch up with the empirical reality, and this accounts for much of the complexity of the argument. One means by which this is done is to test the theory against case studies such as the Cuban missile crisis of 1962 and the nuclear alert during the concluding stages of the 1973 Yom Kippur War. One concept of which Brams appears to be especially proud is that of "nonmyopic calculation" which essentially shows (if I have understood it correctly) how a different outcome is achieved when the belligerents are encouraged to take a long-term view and are not dominated by short-term considerations.

The question, however, is whether empirical reality is driving the theory to explain in complicated ways what might be explained more simply by more straightforward methods, or whether the theory can tell us something new. At times it is indeed illuminating but not to anything like the extent the author appears to believe.

In the end Brams suggests that the opponents act the way they do because of the "rules of the game" rather than because of anything very fundamental in the two sides' make-up. This seems to me to be going too far. It may be that the logic of the situations in which "players" find themselves influences the character of their choices, and the way those choices are made cannot be understood solely by reference to the rules of the game. Moreover, in structuring the games themselves, Brams makes some rather arbitrary judgements on the nature of the choices. For example, over Cuba, the options given for the United States are to blockade or to strike at the Cuban bases, and for the Soviet Union to retain the missiles or withdraw them. This does no justice to the actual range of choices—the United States might have done nothing; the Soviet Union might have escalated matters to take in Berlin. In the three basic issues Brams tackles—deterrence, arm-races and verification—only one dimension of the problem is addressed and the way that the problem itself is defined is controversial. On the whole the proposals at the end of the book are sensible, which gives one greater confidence in the analysis, but they are by no means wholly original and could have been arrived at by simpler methods.

In short Brams has demonstrated his skill as an exponent of games theory, and its continuing if modest value as an aid to working through the logic of superpower conflict. He has been far less successful in justifying the somewhat grandiose claims that he makes for the enterprise. □

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AUTUMN SHOWCASE

Principles of Neural Science

Second Edition

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Checks on the spread

Gordon Thompson

Nuclear Proliferation Today. By Leonard S. Spector. *Vintage, New York: 1985. Pp.478. Pbk \$5.95.*

Non-proliferation: The Why and the Wherefore. Edited by Jozef Goldblat. *Stockholm International Peace Research Institute/Taylor & Francis: 1985. Pp.343. £11, \$19.*

Safeguarding the Atom: A Critical Appraisal. By David Fischer and Paul Szasz. Edited by Jozef Goldblat. *Stockholm International Peace Research Institute/Taylor & Francis: 1985. Pp.243. £16, \$28.*

Most people would agree that the world will be safer if membership of the nuclear weapons club does not grow. Likewise, most seem to think that we would all be better off if the present members were to stop their frenzied arms race.

Thus we are faced with a problem of nuclear proliferation in two basic dimensions — "horizontal", which refers to the acquisition of nuclear weapons by additional countries, and "vertical", which refers to increases in the sizes or capabilities of existing nuclear arsenals. (We owe this terminology to Indian diplomats who, in the 1960s, coined the term "nuclear proliferation" specifically to link the two dimensions.) The three books reviewed here, if taken together, provide an up-to-date and comprehensive picture of the state of nuclear proliferation, although only in its strictly horizontal dimension.

With Leonard Spector's book, the Carnegie Endowment for International Peace (based in Washington, DC) has launched a series of annual reports on the spread of nuclear weapons. *Nuclear Proliferation Today* provides an account of the proliferative activities of eight "problem" countries — Argentina, Brazil, India, Iraq, Israel, Libya, Pakistan and South Africa — together with discussions of the role of China, and of the status of controls and safeguards on nuclear technology. The eight nations, using a variety of approaches to getting the necessary materials and technology, either have nuclear weapons already or are seeking to acquire them. India publicly acknowledged its nuclear test in 1974, although labelling it a peaceful explosion. Israel, which may or may not have conducted a test, is generally believed to have a few tens of nuclear weapons, perhaps kept in a disassembled form in times of peace. The other six nations are in more ambiguous positions or are less advanced in their quest for a Bomb.

Two of the eight countries (Iraq and Libya) have signed the Non-Proliferation Treaty (NPT), although they apparently do not feel bound by its provisions. The others have refused to sign, generally

arguing that the NPT is discriminatory and seeks to perpetuate the dominance of the nuclear powers.

Nations have had a variety of motives for their decisions to sign or to stand aloof from the NPT. For representative groups of countries, these motives are explored in *Non-proliferation: The Why and the Wherefore*, a collection of essays describing the domestic, international and technical factors which brought each country to its decision. The group of NPT non-signatories studied includes two nuclear-weapon states (China and France), all the non-NPT "problem" countries mentioned above, and Spain. Representing the NPT signatories is a group of six countries ranging from Canada (which has never sought an independent nuclear arsenal) to Taiwan (where plans to build a reprocessing plant were abandoned following heavy pressure from the United States in 1976).

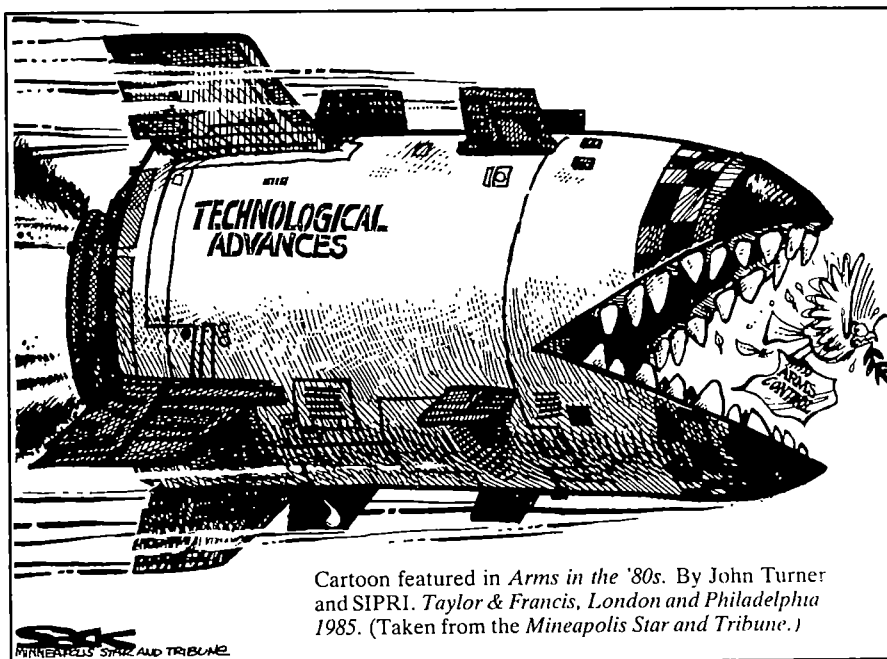
For the book, the Stockholm International Peace Research Institute (SIPRI) has assembled a stable of well-qualified authors (many of them citizens of the countries they discuss) and, to assist in framing the conclusions presented in the overview chapter, the Institute held a workshop in October 1984, attended by a separate group of international experts. Also, SIPRI has drawn on its own resources to prepare several detailed appendices, including one which provides a thorough account of the nuclear infrastructure in each country considered (although the data for China and France are badly out of date).

Comparatively speaking, the conclusions of both SIPRI's study and of Spector's more narrowly focused book are mildly encouraging; that is, the present pace of horizontal proliferation is slow compared with the pace of vertical proliferation. In part, this is because of the emergence of a broad international consen-

sus that the nuclear club should not grow. From this has flowed both moral pressure and the ability to impose an international non-proliferation regime which has complicated the task of any nation seeking nuclear capability. Moreover, the financial costs and technical problems of acquiring an arsenal of nuclear weapons and appropriate delivery systems have proved formidable for developing nations. Finally, growth of the nuclear power industry has been much slower than anticipated; in consequence, possession of a complete nuclear fuel cycle (with the concomitant possibility of diverting fissile materials from reprocessing or uranium enrichment plants) cannot be economically justified for any country, particularly a developing nation.

Central to the non-proliferation regime is the safeguards system operated by the International Atomic Energy Agency (IAEA). This system is the main subject of another SIPRI book, *Safeguarding the Atom: A Critical Appraisal*, which has as its principal author the former IAEA official David Fischer. It also contains a section by Paul Szasz (also formerly with IAEA) and other sections and detailed appendices prepared by SIPRI.

The safeguards laid down by the IAEA are supplemented by other arrangements. First, the Treaty of Tlatelolco, which seeks to make Latin America a nuclear-free zone, is based upon IAEA regulations but also provides for special inspections by a regional agency in the event of suspicious activities. Secondly, the European Economic Community has its own internal system of EURATOM safeguards, which rather wastefully duplicates that of the IAEA. Then there is the Nuclear Suppliers' Group, often known as the London Suppliers' Club, a group of those nations which dominate the export of nuclear materials and technology and which have agreed not to supply certain items



Cartoon featured in *Arms in the '80s*. By John Turner and SIPRI. *Taylor & Francis, London and Philadelphia 1985.* (Taken from the *Mineapolis Star and Tribune*.)

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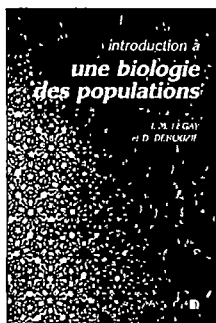


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par E. SHECHTER

1984, 232 pages
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Table of contents

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unless the recipient nations agree to safeguards (the list of sensitive items is similar to the so-called "Zangger Trigger List", drawn up by a committee of nuclear suppliers chaired by Claude Zangger of Switzerland). Finally, there are the arrangements made by individual countries, of which the most important is the United States Nuclear Non-Proliferation Act of 1978.

The NPT and IAEA safeguards, together with these other arrangements, constitute the non-proliferation regime. As mentioned above, the regime has been helpful in restraining horizontal proliferation: much of the nuclear industry outside the nuclear weapon states is under safeguards, as described in detail by David Fischer, while the "problem" countries generally find it difficult to obtain materials and technology for their weapons programmes. Yet, the future stability of the regime is in question.

As both SIPRI books acknowledge, without pursuing the point, the non-proliferation achievements of the past 20 years could be swept away by changing circumstances. First, the NPT remains in force only until 1995 and it is by no means clear that the treaty will be renewed or replaced by a better arrangement at that time. Many signatories are dissatisfied with the failure of the nuclear-weapon parties (the United States, the Soviet Union and Britain) to live up to their side of the bargain — that is, to end their nuclear arms race. Secondly, regional instability could precipitate a rush of nuclear competition between certain powers. Pakistan and India, for example, could fall prey to such competition, and the tension between these two nations illustrates some important linkages of proliferation. American and Soviet nuclear competition has fed the Chinese nuclear build-up. In turn, this has contributed heavily to India's flirtation with the Bomb. Pakistan has reacted, and thereby fed the fears of Israel about an Islamic Bomb. To compound these linkages, China has apparently assisted Pakistan in its quest for nuclear weapons.

An unavoidable conclusion of all this is that nuclear proliferation cannot be considered in just one dimension. Equally, it is hard to envisage any substantial improvement in the horizontal dimension without progress on control of vertical proliferation. In this respect, all three books are deficient. In particular Leonard Spector reflects the view that proliferation is something which other nations do. This attitude, common in the United States, is perhaps understandable given America's role in constantly raising the qualitative level of vertical proliferation. While SIPRI, as befits its fine record of analysing military activities of all kinds, provides a more balanced view, *Non-proliferation: The Why and the Wherefore* does not really pursue the matter of cross-dimensional linkages.

Readers seeking to understand vertical proliferation have the choice of a vast literature, but at some point would be well advised to consult William Arkin and Richard Fieldhouse's *Nuclear Battlefields: Global Links in the Arms Race* (Ballinger, 1985) which provides a succinct summary of how the nuclear confrontations of the five major powers proceed in a myriad of ways. By outlining the vast global infrastructure which supports development, testing and deployment of nuclear weapons, the book makes clear that the implications of vertical proliferation reach further than we might think.

Two examples are instructive. First, through their participation in the Eastern and Western blocs, NPT signatories can function almost as though they had never signed the treaty. Indeed, six NATO countries which are supposedly "non-nuclear" by virtue of being NPT signatories, and four Warsaw Pact countries in the same position, have numerous delivery systems certified to fire nuclear warheads. When needed, the weapons will be released to them by their nuclear-armed allies. Secondly, the intelligence, targeting, basing, training and communications infrastructure for the nuclear arsenals is spread around the globe, taking in many countries which fancy themselves "nuclear-free" and making a mockery of the idea of nuclear-free zones.

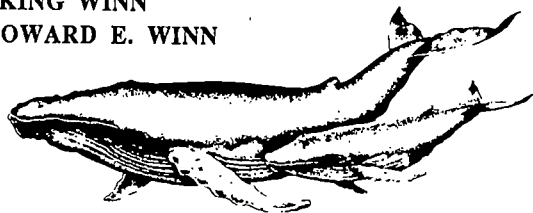
If the non-proliferation edifice begins to crumble, we will rapidly become aware of a third dimension of proliferation. This, the "latent" dimension, refers to the growing ability of many nations rapidly to field a nuclear arsenal should they so decide. The necessary materials and expertise are becoming more generally available as a result of nuclear power programmes. In particular, the wide adoption of nuclear fuel reprocessing and the development of new enrichment technologies imply the stockpiling of large quantities of fissile materials. Despite the lack of economic justification, industrial nations, including Japan and West Germany, are pressing ahead in these areas. Since appropriate nuclear weapon delivery systems are readily available, such nations could field large arsenals with surprising speed. But again, this possibility is discussed in none of the three books reviewed here.

Viewed from a wider perspective, then, nuclear proliferation is a far more serious problem than the strictly horizontal trends of recent years might suggest. It is not alarmist to say that we urgently need to tighten international controls on all forms of proliferation. The current non-proliferation regime provides a basis for doing this, but the world community needs to find the vision to adopt substantial reforms. □

Gordon Thompson is Director of the Institute for Resource and Security Studies, 27 Ellsworth Avenue, Cambridge, Massachusetts 02139, USA, and coordinator of the IRSS Proliferation Reform Project.

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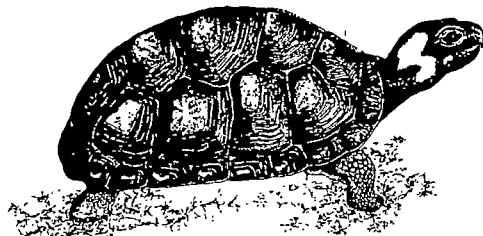


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Towards a New World synthesis

Colin A. Russell

Chemistry in America, 1876–1976. By Arnold Thackray, Jeffrey L. Sturchio, P. Thomas Carroll and Robert Bud. Reidel: 1985. Pp.564. Dfl.210, \$79.50, £53.50.

A CASUAL reader who decides to browse through this volume is in for quite a surprise. From the title one might expect an account of American chemistry (here meaning chemistry in the United States) on the lines of those engaging and once-popular series "a hundred years of this or that": bland, popular, anecdotal, synoptic, well-illustrated, easy on the eye and reasonably analytical. For the price and size one might expect all this on a lavish scale. Should our browser dally even for a moment at the title page he will, however, receive the first hint of unpredictability. The subtitle "historical indicators" suggests uncharted seas ahead. For, if these are not to do with the litmus-type indicators familiar to all chemists, what on earth are they? The confusion is not resolved by the apparently perverse and deliberate appropriation of the very phrase "chemical indicators" that has for so long had a quite precise if pedestrian connotation of an entirely different kind.

"Indicators", as used by these authors, are long-running trends in a series of statistical data. They are measurements of change. If, for example, you analyse census and other information about the employment of chemists in the United States, you find for the past century or so an annual growth rate of about five per cent in the chemical work-force, and that chemists have increased from 2 to 15 per 10,000 of the total numbers employed in any work. On the other hand, the proportion of high school chemical students who later specialize in chemistry has declined steadily, as has the esteem accorded to chemistry in public opinion surveys and its coverage in newspapers. And since about 1910 there has been absolute growth *but relative decline* in the place of chemistry in the internal economy of American universities.

In six chapters, occupying less than half the book, the authors present a profusion of tables, graphs, bar-charts and the like to show how American chemistry has changed in the context of industry, education and its professional organization. Their conclusions are helpfully summarized in each chapter by a few "indicator highlights". For people who are prepared to take the arguments on trust these brief conclusions are probably all that matter: two or three pages of print altogether. However with typical trans-Atlantic thoroughness the authors will not allow

us off the hook so easily. They parade their hard-won information in a spirit of modesty and self-criticism, and delight in warning of the hazards and limitations of their approach. As if to hammer the point home they append to the book over 200 pages of tables, some appendices on methodology and a bibliography of massive proportions.

The methods, like the idea of "indicators", derive from the social sciences. So also, apparently, does the phraseology, though this is mainly evident when the authors are being self-consciously coy about their strategy. Indeed it seems that the most avid users of the book are likely to be those students of society for whom chemistry is but another manifestation of social conditioning and manipulation. Such enquirers will find a wealth of raw material brought together for the first time, and also many hints and ideas for future research. Yet it is as part of a series devoted to the history of chemistry that this book appears, so it is not unreasonable to enquire what chemists and historians of science might make of it.

Chemical readers will here find little of the *content* of their science, even historically treated. They will, however, discover a great deal about its *context*, in which only the most myopic benchworker will be altogether uninterested. The conclusions, at least for American chemists, are not wholly agreeable, for the subject's decline is remorselessly depicted in the many graphs and tables. For those who plan and teach, these trends, however depressing, cannot now be ignored. If, as the authors claim, "chemical education begins to look like a sieve, leaking credentialed chemists in all directions", one would have thought that those who advise American youth would at least be glad to know. So, one imagines, would those responsible for science policy.

For historians of chemistry, the book will have little appeal to those who like their history warm, human, personalized and cosy. Nor will it be of much use to their polar opposites, historians of scientific ideas, philosophies and techniques. People to whom quantitative historical science is a contradiction in terms ("history by numbers" such efforts are contemptuously called) will look elsewhere for inspiration. Nor must the sceptics be dismissed as mere backwoodsmen. They have a real point when they urge that huge compilations of statistics either prove what you want them to prove (just select the right parameters) or that they merely clothe the blindingly obvious with pseudo-quantitative respectability. It is to the great credit of the authors of this book that they are well aware of these dangers, and do not attempt to conceal them and can (in a couple of apposite cartoons) even laugh at themselves.

Those whose interests lie in the so-called social history of science will find in *Chemistry in America* a fund of good ideas

and useful information, especially if they are concerned with institutions or education. Yet the book also has drawbacks for such people. The material relates to only one country — though admittedly a world leader in chemical research — and one is bound to seek comparisons with Britain, Germany and other countries if only for the earlier part of the period under consideration. Moreover, it is important that not only should national trends be compared but that allowance be made for different meanings for the variables measured. Thus the criterion of professionalization in chemistry is a very American one and different from that in Britain where the phenomenon first arose. Similar caution must be given about the definition of various classes of chemists which mean different things in different countries. "assayer" is a case in point.

It is not difficult to criticize the selection and presentation of the data. One looks in vain for information on such topical issues as the changing role and numbers of women in chemistry, the relation between chemistry and war, and even the question of chemists' salaries. In education, trends in chemistry are compared with those in history rather than in physics and biology. Again, there are surely more important issues to record than the number of ex-chemists who become deans in American universities or the ages (high) of Presidents of the American Chemical Society. However, the prominence given to that specific institution is one of the particularly creditable features of a book that is always in danger of submersion in an ocean of generalities.

In reading the book I became gradually conscious of a sense of *déjà vu*. Somewhere I had met something very like it before, but where? The impression crystallized into recognition by about Chapter 3. Its predecessor was nothing less than the massive *History of Chemistry* by J. R. Partington, published by Macmillan in four volumes between 1961 and 1970. Superficially the two works are light-years apart yet fundamentally they are doing the same thing: presenting a highly idiosyncratic view of a mountain of information with little or no attempt to digest it into a coherent, readable overview of the subject. Here, perhaps, is a Partington of a new era, with all the weaknesses and strengths of the original. Indigestible it may be, and one could certainly not read it from cover to cover. But if it turns out to be half as useful as Partington in supplying raw information and in catalysing future work, it will earn the gratitude of generations to come. Whether the trends it heralds in chemical history — empirical, pragmatic and semi-quantitative — will prove to be fruitful, time alone will tell. □

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Space shuttles

Peter Kemp

Contact: A Novel. By Carl Sagan. *Simon & Schuster: 1985. Pp.432. \$18.95.*

AMONG the numerous — and often numinous — quotations strewn around Carl Sagan's latest novel, is Einstein's claim that "the cosmic religious feeling is the strongest and noblest motive for scientific research". True to this belief, Sagan's saga of galactic exploration keeps juxtaposing the scriptural and the scientific: apocalyptic sects and astronomers, angels and astronauts, messiahs and supernovae, tales of mystic experience and talk of transcendental numbers. Even in shape — opening with genesis and ending with revelation — his book seems to have its eye on the Bible.

The first page records the birth of Ellie, a baby who comes into the world with a look of "puzzlement". As she grows up, this matures into intellectual curiosity — especially about possible extraterrestrial life. Finding her vocation as a radioastronomer, Ellie pioneers new techniques for scanning the heavens — and, as the second millennium approaches, her hunch that there are powerful beings out there proves justified. From the vicinity of Vega, a coded communiqué is picked up, transmitting instructions for the manufacture of a massive machine. Despite objections from some quarters that this is a snare of Antichrist, construction work goes ahead. At last — on 31 December, 1999 — Ellie and four international colleagues board what has turned out to be a spaceship ready for launching.

Up to this point, Sagan's story has kept to fairly well-trodden ground, such as the way space research might foster "transnational" thinking and global unity: a notion regularly mooted in science fiction since the days of H. G. Wells. The crucial test for space travel stories, though, is what happens when their characters zoom away from Earth. After take-off, the narrative is at maximum risk of nose-diving into the banal.

This is a hazard Sagan doesn't altogether steer his way around. Though he musters an imposing array of technical terminology — as his crew shuttle through space, they exchange arcane remarks about "time-like Killing vectors, non-Abelian gauge invariance, geodesic refocusing, eleven-dimensional Kaluza-Klein treatments of supergravity" and the like — he also employs analogies that seem something of a let-down. The mysterious black tunnels along which his starcraft is whisked are, it is improbably suggested, like "a subway"; star systems loom up like stops along the line; the gigantic junction at the heart of it all is "Grand Central Station".

Even more surprisingly down-to-earth

is the destination at which his travellers disembark: a kind of far-fetched Disneyland. Alighting on a simulation of a tropical beach — all palm trees and balmy breezes — they are greeted by aliens who have considerably assumed not merely human shape but that of their guests' "deepest loves". More suggestive of *When You Wish Upon A Star* than of what you might expect to find when you travel to one, these sentimental look-alikes are also designed, it appears, to figure as equivalents of scriptural divinities: "beings who live in the sky, beings enormously knowledgeable and powerful, beings concerned for our survival". "How . . . theological

. . . the circumstances had become". Ellie reflects excitedly. But, for all the book's strainings towards some loftily spiritual sphere, its final message is mundane. Returned to Earth after her galactic gallivantings, Ellie clutches the insight that "For small creatures such as we the vastness is bearable only through love". A piece of enlightenment you'd hardly think it necessary to travel light-years to acquire, it's disappointingly typical of the way this story tends to drop from the nebular to the nebulous. □

Peter Kemp is an Associate Lecturer in English at the Middlesex Polytechnic, All Saints, White Hart Lane, London N17 8HR, UK.

A light on the past

Hendrik B.G. Casimir

History of Physics: Readings from Physics Today. Edited by Spencer R. Weart and Melba Phillips. *American Institute of Physics: 1985. Pp.375. Pbk \$25.*

WHEN, in 1948, the American Institute of Physics (AIP) launched *Physics Today*, it did not intend the journal to deal with the past. The new publication addressed itself to members of the AIP and of its several affiliated bodies, and aimed at keeping them informed about what was happening in physics, both from a scientific and from an institutional point of view. Only in 1952 did a contribution of a historical, or at least of a retrospective character appear in its columns, but from then on historical articles became a more or less regular feature. *History of Physics* — a somewhat grandiose title which is put into proportion by the inconspicuously printed subtitle, *Readings from Physics Today* — is an anthology of such articles, together with a brief introduction.

The introduction gives a useful survey of the activities of the AIP in the field of history and of the development of other such work in the United States since the Second World War. In another respect, however, it is not entirely satisfactory; when the editors state that there was hard-

ly any organized research into the subject before 1950 — that there were no books, no papers, no journals on the history of physics — they are taking a rather parochial view. On this side of the Atlantic we had E.T. Whittaker's great work *History of the Theories of Aether and Electricity* and the famous *Ostwald's Klassiker*, annotated reprints of important papers, to name but two examples. Moreover there were many biographies and autobiographies, and several journals dealing partly or entirely with the history of science, including physics.

This, however, is a point of minor importance and should not deter prospective readers. What really matters is the selection of material and in this respect the editors have done an excellent job. Not surprisingly, the articles differ widely in style and in scope, which makes it difficult to characterize the book as a whole. The best I can do is to discuss a few typical examples.

Two papers, one by Goudsmit and one by Uhlenbeck, deal with the birth of the notion of the spinning electron and with the circumstances that surrounded it. Here we have the story of a seminal idea, related by the originators half a century later. In consequence not only do these essays give a vivid impression of quantum physics and spectroscopy in the 1920s, and of the little group working with Ehrenfest at Leiden University, but they also provide an intriguing insight into the later

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NATURE

[FEBRUARY 20, 1926]

Letters to the Editor.

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Spinning Electrons and the Structure of Spectra.

So far as we know, the idea of a quantised spinning of the electron was put forward for the first time by A. K. Compton (*Journ. Frankl. Inst.*, Aug. 1921, p. 145), who pointed out the possible bearing of this idea on the origin of the natural unit of magnetism. Without being aware of Compton's suggestion, we have directed attention in a recent note (*Naturwissenschaften*, Nov. 20, 1923) to the possibility of applying the spinning electron to interpret a number of features of the quantum theory of the Zeeman effect, which were brought to light by the work

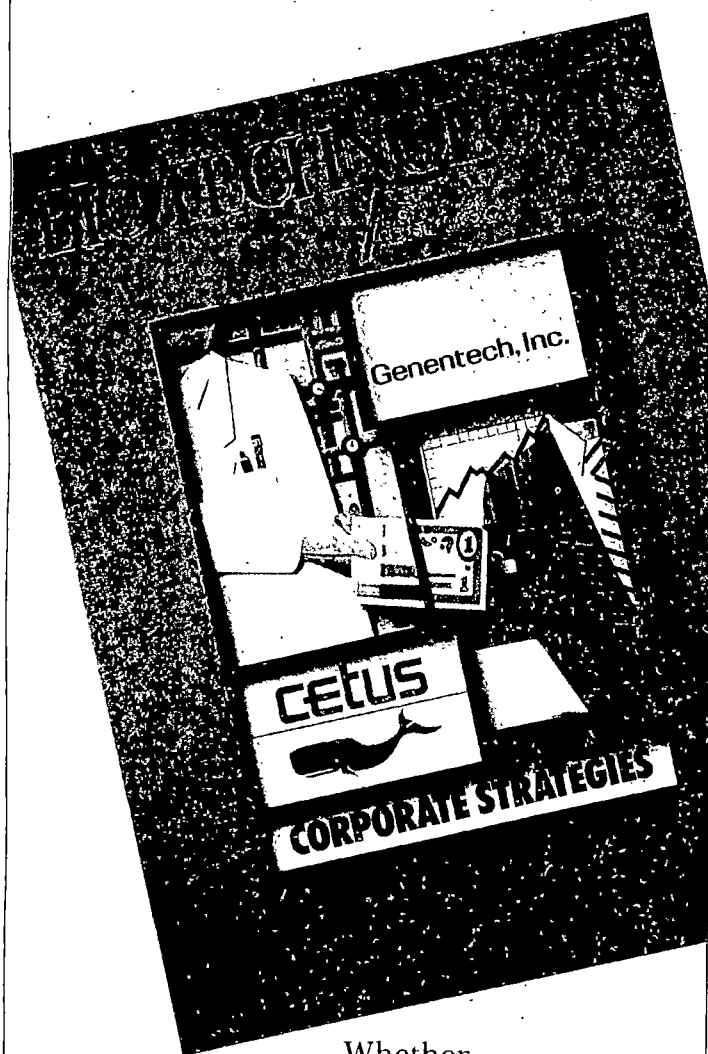
of this moment of momentum is given by $Kh \cdot 2\pi$, where $K = \frac{1}{2}, 1, \frac{3}{2}, 2, \dots$. The total angular momentum of the atom is $Jh \cdot 2\pi$, where $J = 1, 2, 3, \dots$. The symbols K and J correspond to those used by Landé in his classification of the Zeeman effects of the optical multiplets. The letters S, P, D also relate to the analogy with the structure of optical spectra which we consider below. The dotted lines represent the position of the energy levels to be expected in the absence of the spin of the electron. As the arrows indicate, this spin now splits each level into two, with the exception of the level $K = \frac{1}{2}$, which is only displaced.

In order to account for the experimental facts, the resulting levels must fall in just the same places as the levels given by the older theory. Nevertheless, the two schemes differ fundamentally. In particular, the new theory explains at once the occurrence of certain components in the fine structure of the hydrogen spectrum and of the helium spark spectrum

k old K new J

Birth of the notion of the spinning electron

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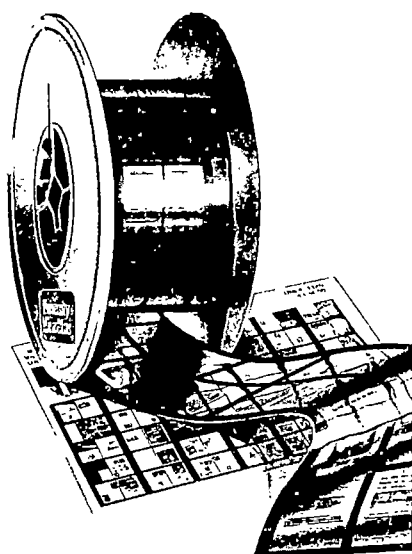
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views of two senior scientists.

A delightful curiosity is the text of an address given by Einstein at Kyoto University on 14 December 1922, "How I Created the Theory of Relativity". The lecture was given in German and a Japanese physicist, J. Ishiwara, provided a translation. In 1923 Ishiwara published his Japanese text and in 1982 *Physics Today* printed an English translation by Yoshimasa A. Ono. Clearly, knowing that the lecture was to be translated, Einstein had formulated his ideas as concisely and simply as possible, but even so it is remarkable to see how well the text has survived two consecutive translations. There are several other articles in which the story of an important discovery or invention is told by people who were not necessarily the only originators but who did play an important part — Frisch and Wheeler on the discovery of fission is a good example, as is the history of the cyclotron told by Livingston and McMillan.

Also included are several biographical essays. Most of these are written by authors who were well-acquainted with their subjects, as their students or collaborators, which makes the stories lively and readable — here, Oliphant's "The Two Ernests" (Rutherford and Lawrence) is especially illuminating. Quite different again are the scholarly papers on specific aspects of the history of physics, for example Martin Klein's thorough study "Thermodynamics and Quanta in Planck's Work". Klein shows how Planck, a conservative rather than a revolutionary, found it hard to reconcile himself to his own innovation, but finally arrived at the conclusion "that the quantum of action played a far more significant part in physics than I had originally been inclined to suspect".

It is understandable that the contributions on institutional history deal almost exclusively with American establishments, and the same is true, though to a lesser extent, of the articles grouped under the heading "Social Context". But this does not mean that they are of no interest to non-Americans, and in many cases the problems discussed are international in nature. Of particular interest here is the article by Vera Kistiakowski with the provocative title "Women in Physics: Unnecessary, Injurious and Out of Place?"

Few people will read this book from cover to cover — the editors even warn against trying to do this. But all physicists and many non-physicists will enjoy leafing through it, will admire the many excellent illustrations, and will sooner or later start reading in earnest. □

Hendrik B.G. Casimir, *De Zegge 7 5591 TT Heeze, The Netherlands, studied theoretical physics at Leiden, Copenhagen and Zürich during the 1920s and 1930s and later became joint director of research at the Philips Company, Eindhoven. His autobiography, Haphazard Reality: Half a Century of Science, was published by Harper & Row in 1983.*

In the realm of the cosmocrats

Edward Harrison

The Cosmological Distance Ladder: Distance and Time in the Universe. By Michael Rowan-Robinson. W.H. Freeman: 1985. Pp.353. \$35.95, £19.95.

COMTE de Buffon once said that the forms of life seem more like chain mail than a Great Chain of Being. Similarly the measure of the universe seems more like a network of ladders than a single cosmological distance ladder. (An irreverent person might say more like a game of snakes and ladders.)

The scale of the universe has been increased several times since Hubble wrote *The Realm of the Nebulae* in 1936. The Hubble term H in the velocity-distance law (velocity = $H \times$ distance) has decreased in value by an order of magnitude and now stands at either 100 or 50 km s⁻¹ Mpc⁻¹. The de Vaucouleurs school champions the higher value and the Sandage-Tammann school the lower value. The reciprocal of H is about fifteen thousand million years and serves as a rough measure of the age of the universe.

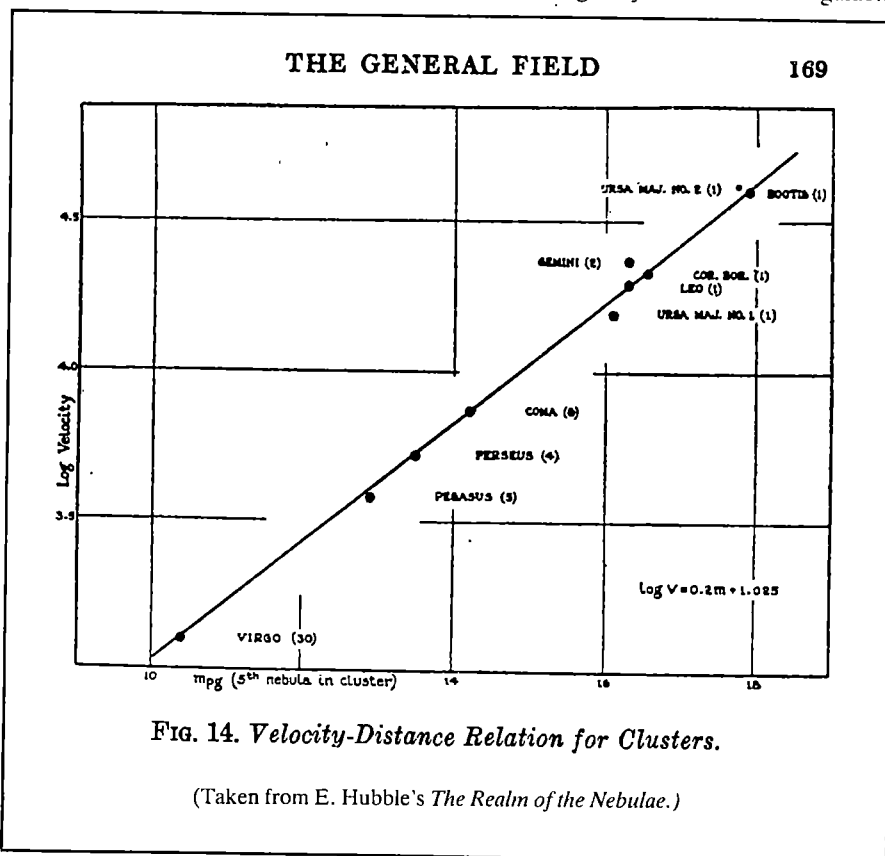
Rowan-Robinson unifies his splendid and comprehensive survey of the universe — planets, stars, star clusters, galaxies, galaxy clusters, superclusters — with the theme of the cosmological distance ladder. In the preface he writes that, at a conference in 1976,

Gerard de Vaucouleurs on the one hand, and Allan Sandage and Gustav Tammann on the other, arrived at estimates of the size of the universe, as measured by the Hubble constant, differing from each other by a factor or two. Moreover, when I asked the protagonists what was the range outside which they could not imagine the Hubble constant lying, these ranges did not even overlap. . . . It was to understand this disagreement that I set out to write this book.

Using numerous figures, tables and appendices that display a wealth of data, the author builds an array of distance indicators and presents a difficult subject in a methodical manner. Skilfully he adjusts, deftly weighs, and adroitly averages divergent results.

The first rung on the ladder is the distance between the Sun and the Earth. Then comes the distance to the Hyades, the nearest open star cluster, determined by parallax and moving cluster methods. The size of this preliminary and important step, now estimated to be 150 light-years, has grown 30 per cent since 1940.

We measure in Hyades-units the distance to other open clusters, applying various methods including evolutionary curve fitting. Then, with allowance for absorption of light by interstellar dust, we use Cepheids and RR Lyrae variable stars as yardsticks to gauge the distances of globular clusters in our Galaxy and of nearby galaxies in the Local Group (rival schools going different ways and making different allowances for extinction of light). Novae, supernovae and HII regions, adjusted for extinction in the observed galaxy, serve as intergalactic



indicators of distance; here the methods become less reliable and the uncertainties increase yet further. Supergiant galaxies then act as stepping stones to remoter regions.

It began with the Greeks. Rowan-Robinson gives them a page or two and then jumps, as one expects in an astronomer's history of astronomy, from Aristotle to Copernicus. The Epicurean system (an infinite atomic universe) and Stoic system (an island cosmos surrounded by an infinite extracosmic void) receive scant attention, although both dominated the intellectual climate of the ancient world. The great historical drama of the mediaeval revival of the Aristotelian system, the eclipse of this system in the late Middle Ages by a nascent Stoic system, the rebirth of the Epicurean system, and the eventual overthrow of the Stoic system in the Great Debate of the 1920s also receives little recognition. We are told that in the seventeenth century Cassini measured the Sun–Earth distance to within 10 per cent of the modern value, but not that Newton, by using James Gregory's photometric method of comparing the outer planets with the brightest stars, determined with astounding accuracy (within a factor of two for Sirius) the distance of the nearest stars.

In a chapter entitled "The Cosmological Models", which is devoted to theoretical topics, the author discusses dynamic models, redshifts, proper and luminosity distances, the early universe, and the motion of the Solar System relative to the microwave background and to the Local Supercluster. Perhaps more such analysis in the other chapters would have helped the reader to surmount the mountains of data to be found in them.

The axioms or irreducible assumptions of modern cosmology rarely receive adequate attention, and the book would have benefited from some discussion of these hidden assumptions. The Hubble (and deceleration) term tell us how the universe expands. But how exactly do we need to know its value? Theorists first postulated the velocity–distance law, which applies at any instant throughout a homogeneous expanding universe. But when we look out in space we also look back in time, and homogeneity (unlike isotropy) cannot be directly seen. Observations made on the backward light cone are adjusted for evolutionary effects and mapped in a hypothetical homogeneous space. At best we verify that redshifts and estimated distances are consistent within the theoretical frame of a geometric and dynamic model that is itself unverified. Perhaps our models are too simple and we do not know enough cosmology to make an exact value of the Hubble term meaningful? □

Edward Harrison is Professor of Astronomy in the Department of Physics and Astronomy, University of Massachusetts, Amherst, Massachusetts 01003, USA.

Halley's comet in print

David W Hughes

ON 16 October 1982 Halley's comet was picked up using a charge-coupled device at the prime focus of the 5.1 m Hale telescope on Mount Palomar. It has been monitored ever since. As it approaches the Sun it brightens and will become just visible to the naked eye between December 1985 and April 1986. But there are two problems for observers. On 9 February 1986 the comet is at its closest to the Sun and for a six-week period around then is in too bright a region of sky to be easily visible. Also, between December and April it sweeps from the northern to the southern sky, requiring the northern observer to reciprocate by moving to the southern hemisphere. Unfortunately planet Earth does not get very close to the comet this time, so we are not going to be overawed by startling searchlight-like beams radiating up from the twilight horizon. In fact the mere act of catching sight of the comet will require some effort.

Halley's return to the inner Solar System has been greeted by a whirlwind of books, and thus a rather rare state of affairs as far as the reviewer is concerned. Most of them in fact say much the same things, use the same illustrations and are aimed at a like audience. Most are written by non-specialists who, for simplicity, have consulted only secondary sources of information and have consequently made many mistakes. Reviewing them is akin to essay-marking. To help the prospective buyer the books are divided into categories, with a star rating for each according to whether it is thought to be excellent, good, indifferent or poor. The five-star rating is reserved for those rare books in the pile that will "last", to be consulted around AD 2061 when Halley's comet returns once more.

The categories are as follows: (i) Books specifically designed to help the reader see the comet (the message being that you have a once-in-a-lifetime chance so make the most of it); (ii) guides for teachers, that is books specifically designed for the organizers of classes; (iii) specialist books; and (iv) general books.

Halley's Comet by Francis Reddy leads the first category. It abounds with good advice: "If you want to see the comet you had better prepare for it, familiarize yourself with the constellations through which Halley moves, get away from cities, use your binoculars and travel south". Twenty maps are provided, for three Earth latitudes (55°N, 40°N and 35°S). These not only show the comet and its tail against the starry background but also include (where appropriate) the Moon, Mars and Jupiter. A superb three-dimensional model of the orbits of the comet and Earth is an additional bonus.

Such models are a great help when it

comes to visualizing the movement of the comet with respect to the Sun and Earth, and Perihelion Scientific have produced one, *Halley's Comet Survival Kit*, which is 85 cm across. Another accompanying model enables you to calculate the altitude and azimuth of the comet throughout the November 1985–May 1986 period. Both are great fun to assemble and use.

If you are happier with a planisphere, David Chandler has designed a set of three (for northern latitudes 26°, 35° and 44°). These show the comet's path through the constellations and it is simplicity itself to calculate when and where it can be seen. Unfortunately there are no hints as to its brightness.

Halley's Comet Finder by Ben Mayer is aimed more at the astronomically initiated and gives a detailed review of the comet's journey across the sky. The section on astrophotography in this book is most useful.

For the complete beginner the editors of *Sky and Telescope* have produced *Mr. Halley's Comet* (I've no idea why they refer to a man who was an eminent professor and had two doctorates as "Mr."). This is an excellent and inexpensive guide marred only by the sparsity of stars on the sky map. The fact that a pair of binoculars has only a limited field of view is rightly stressed, but the difficulty of finding the comet and recognizing stars in the field is somewhat overlooked. The guide seems to be designed in the hope that the comet would be much brighter.

Moving into the classroom, both *Comet Halley Returns* and *Halley's Comet Activities Manual* get good marks and will provide science teachers and their scholars with hours of activity understanding comets, modelling their orbits, recording observations, computing examples of Keplerian motion, introducing the life and times of Edmond Halley and trying to appreciate his achievement. *Halley's Comet: Teacher's Notes* comes with posters and is biased towards young (9–14) or innumerate pupils (and supposedly innumerate teachers too!). It approaches cometary science from a sociological and refreshingly critical standpoint — I'm still considering "the possibility that women astronomers do not appear in astronomy books because those books are generally written by men", one of the topics suggested for classroom discussion. Mary Ashby, the author, has a clear grasp of what children find interesting and what they find difficult to understand, and has planned a series of exercises for explaining difficult concepts.

Nine books fall into the specialist category, two of which are bibliographies. Ruth S. Freitag lists 3,289 references to both popular and scientific cometary literature. The list is alphabetical by author and each

reference is followed by a short description of the item. This book is an indispensable source for anyone seeking data on the comet's previous apparitions, technical literature on the Halley spacecraft missions or manifestations of popular interest in comets. Just turn to the index, and look up, for example, "Essays and sketches, humorous"; "fiction"; "recovery (1759)"; "transits across the sun's disk"; "music"; and "mass spectroscopy". What a godsend this book is. I hope that Ruth Freitag can be persuaded to bring the work up to date in, say, 1990 when the present hullabaloo has died down.

Bruce Morton's bibliography will be less useful because he has deliberately omitted all non-English language source material. The listing is chronological but is backed up with an author and subject index. Morton only lists 1,301 items but does include many not covered by Freitag. Researchers will need both books.

Fire and Ice by Roberta J.M. Olson is an art historian's view of comets and has been published to accompany an exhibition of comets in art at the National Air and Space Museum in Washington, DC. The main attraction of Olson's book is the art reproductions. There are over a hundred pictures of comets, many in colour. Rackham, Moreau, Turner, Gérard, Blake, Daumier, van Stolk, Stech, Scott, Giotto — the list of artists is impressive. The text serves two purposes, first, to introduce the pictures and fit them into the context of the history of art, and secondly to weave in the development of man's fear and understanding of comets as shown in the paintings, engravings and illustrations of the day. All this is both entertaining and enlightening, but let me mention two minor quibbles. If someone has gone to the trouble to paint "Dr Halley" across the top of the Phillips portrait of our hero, why entitle it "Sir Edmund Halley" in the book? He wasn't knighted and he spelt his christian name *Edmond*. Also it must be remembered that Halley's comet was not *that* famous before 1705. Giotto might not have had to remember the comet of 1301; he might have popped out in December 1304 and painted the long-period comet that was in the sky then.

Peter Lancaster-Brown in *Halley and his Comet* has concentrated on the non-artistic historical aspects of the story, and creatively charts the transition of comets from astrological portents (in pre-Halleyan times) to their present status as unusual astronomical objects. The author has produced a most readable book, but one in which he has not resisted the temptation to fill in some of the gaps: lines such as "We might surmise without stretching credulity . . ." often end in rather too imaginative conjecture. If Newton was a latent homosexual, and Halley a philanderer, I would like chapter and verse.

All serious cometary historians should get a copy of *La Comète de Halley: Hier,*

Aujourd'hui, Demain by J. Alexandre and S. Debarbat. This is a small folder containing a collection of reproductions of Halley comet documents from the Paris Observatory. The Lubientietz maps and the drawings by Hevelius, Arnold, La Hire, J.-D. Cassini and Schwabe are fascinating, while Messier's hand-written account of the 1758 apparition is in the first division of historic documents.

Comet Fever by Donald Gropman has as its main theme the 1910 appearance of the comet and especially the dramatic events of the night of 18–19 May when the Earth swept through the comet's tail. We are told of sealed windows, absenteeism from home and work, mass prayer ses-



Richard Phillip's portrait of Halley circa 1721.

sions and, to cap it all, all the news fit to print about the infamous Oklahoma 19-year-old-naked-virgin-sacrifice-drama. Other chapters skim over past apparitions of Halley and aspects of modern cometary science, making in all a well-written, extremely readable book.

Fred L. Whipple is the doyen of comet scientists having worked extremely profitably in the field of cometary astronomy for 50 years. What he has to say about comets is certainly worth reading and his book, *The Mystery of Comets*, is superb. Too few scientists take the time to explain the complexities of their subject to a general audience. Whipple has left nothing out — if you want a synopsis of what is known about comets and, even more exciting, what is still beyond our present grasp, this is the book for you.

Another book that will be consulted for decades is *Halley's Comet in History*, edited by F.R. Stephenson and C.B.F. Walker, which emphasizes the Babylonian and Chinese records of the comet between 240 BC and AD 1531. The 13-page, line-by-line translation of a 164 BC Babylonian cuneiform tablet is rather strong meat for the casual browser, but if you need the historical information this is where to look. I wish more than one figure showing the comet's path across the sky had been included — the book would have been greatly enhanced by including a set of these for the relevant 23 apparitions.

Comet by Carl Sagan and Ann Druyan is a visual and intellectual feast, combining a host of specially commissioned colour illustrations with a text that, for the breadth of insight into the past, present

and future of cometary science, is way ahead of the rest of the field.

Standing back from all the razzamatazz surrounding this apparition, the book concentrates on the fundamentals: why have comets worried people, what have scientists and philosophers made of them, what is their origin, composition and fate? The Sagans' inquisitive enthusiasm is infectious. Sometimes they approach that danger zone where the gap between fact and speculation is overlooked, but they are soon forgiven. For me, comets will never be the same again. Cometary science can only blossom with such champions.

Finally, to the general books — those containing a bit of everything, history, comet lore and an observer's guide. Confronted with eight of them the reviewer is forced to list them in order of preference, a difficult task because they all have "curate's egg" properties. First past the post is Patrick Moore and John Mason's *The Return of Halley's Comet*. The maps are excellent, giving you a real chance of spotting the comet, and the review of comet science and decay is impressive. Make sure you get the new edition.

Close behind are Brian Harpur's *The Official Halley's Comet Book* and Mark Littmann and Donald K. Yeomans's *Comet Halley: Once in a Lifetime*. Both are superbly illustrated and overflowing with anecdotes. Harpur's review of cometary poetry and literature is masterly, while Littmann and Yeomans put across the scientific message superbly. With both, however, you will need additional help to find the comet.

In much the same category we find *A Comet Called Halley* by Ian Ridpath and Terence Murtagh. The text is excellent but some of the illustrations are a touch odd; surely the *Vega* spacecraft doesn't look like that! And it would be much easier to find the comet if it was shown with respect to compass points and elevation, and not just vaguely trundling across the constellations.

Occupying the uninspiring section where the errors proliferate and the illustrations become even more blurred we find four books by Richard Flaste *et al.*, John Tullius, Donald Tattersfield and Isaac Asimov. Reading these convinces me that there are many better ways of spending one's time and money.

The entire collection (which, incidentally, is by no means a comprehensive display of Halleyana) is listed in the table on the following page. But, to summarize: if you want to see Halley's comet read Francis Reddy; if you want to stay in your armchair with your feet up, read Brian Harpur, and Sagan and Druyan; if you want to know about comets in general you can do no better than Fred Whipple; and if you want to find something out for yourself get Ruth Freitag's bibliography. [.]

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Halley's comet in print — details of the publications

Title	Author/editor	Publisher	Pp.	Price	Rating
Seeing the comet					
Halley's Comet	Francis Reddy	AstroMedia, Milwaukee, WI/Pan, London	59	Pbk \$9.95, £7.95	*****
Halley's Comet Survival Kit	Tom Gaffney <i>et al.</i>	Perihelion Scientific, Paoli, PA	—	North America \$14.95; elsewhere \$20.25	**
The Night Sky Planisphere with Halley's Path	David Chandler	Sky Publishing, Cambridge, MA	—	\$5.95	**
Halley's Comet Finder	Ben Mayer	Perigee, New York	77	Pbk \$5.95	**
Mr. Halley's Comet: Everyone's Complete Guide to Seeing the Celestial Event	Editors of <i>Sky and Telescope</i>	Sky Publishing	32	\$2	**
Guides for teachers					
Comet Halley Returns: A Teachers' Guide 1985–1986	R.O. Chapman and R. Lynn Bondurant, Jr	NASA, EP 197	41	Pbk \$2	****
Halley's Comet Activities Manual	George S. Mumford	Sky Publishing	41	\$4	****
Halley's Comet: Teacher's Notes	Mary Ashby	Inner London Education Authority	53	£5.50	**
Specialist books					
Halley's Comet, a Bibliography	Ruth S. Freitag	Library of Congress, Washington, DC	555	\$26	*****
Halley's Comet, 1755–1984: A Bibliography	Bruce Morton	Greenwood Press, Westport, CT/Eurospan, London	—	\$35, £43.50	**
Fire and Ice: A History of Comets in Art	Roberta J.M. Olson	Walker, New York	144	Hbk \$24.95; pbk \$14.95	***
Halley and his Comet	Peter Lancaster-Brown	Blandford, Poole, Dorset/Sterling, New York	186	£9.95, \$12.95	**
La Comète de Halley: Hier, Aujourd'hui, Demain	J. Alexandre and S. Debarbat	Observatoire de Paris	—	FF 22	****
Comet Fever: A Popular History of Halley's Comet	Donald Gropman	Fireside (Simon & Schuster)	189	Pbk \$7.95	**
The Mystery of Comets	Fred L. Whipple	Smithsonian Institution Press/Cambridge University Press	276	Hbk \$24.95, £12.95; pbk \$12.50	****
Halley's Comet in History	F.R. Stephenson and C.B.F. Walker	British Museum Publications, London	64	Pbk £5.50	**
Comet	Carl Sagan and Ann Druyan	Random House/Michael Joseph	240	\$24.95, £12.95	****
General books					
The Return of Halley's Comet (new edition)	Patrick Moore and John Mason	Patrick Stephens, Wellingborough, Northants, UK/Warner, New York	128	Pbk £4.99, \$6.95	***
The Official Halley's Comet Book	Brian Harpur	Hodder & Stoughton, London/David & Charles, North Pomfret, VT	184	£8.95, \$15.95	**
Comet Halley: Once in a Lifetime	Mark Littman and Donald K. Yeomans	American Chemical Society, Washington DC	175	North America hbk \$19.95, pbk \$12.95; elsewhere hbk \$23.95, pbk \$15.95	**
A Comet Called Halley	Ian Ridpath and Terence Murtagh	Cambridge University Press	48	Pbk £2.95, \$4.95	**
The New York Times Guide to the Return of Halley's Comet	Richard Flaste <i>et al.</i>	Times Books, New York	244	Hbk \$16.95; pbk \$7.95	*
The Science Digest Book of Halley's Comet	John Tullius	Avon, New York	136	\$9.95	*
Halley's Comet	Donald Tattersfield	Basil Blackwell	164	£6.50, \$12.95	*
Asimov's Guide to Halley's Comet: The Awesome Story of Comets	Isaac Asimov	Walker, New York	118	\$12.95	*

Recent progress and future plans on the search for extraterrestrial intelligence

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The possibility of life in other parts of the Universe has long occupied the human mind, but actual searches only began in 1960 with Project OZMA conducted by Frank Drake. In the past 25 years, we have made impressive progress, and this new field has gained broad scientific recognition including the support of the US and the Soviet National Academies, and the endorsement of the International Astronomical Union.

PEOPLE have been fascinated by the possibility of intelligent life in other parts of the Universe for at least 2,500 yr (refs 1, 2). Around 400 BC, for example, the Greek philosopher Metrodorus of Chios wrote³: "It seems impossible in a large field to have only one shaft of wheat and in the infinite Universe only one living world." The question, however, remained a philosophical one until the middle of this century, when Cocconi and Morrison⁴ urged the scientific community to undertake radio searches in the vicinity of the 21-cm line of atomic hydrogen, the only radioastronomical line known at the time, for radio messages from other civilizations. They closed their paper with the statement: "The probability of success is difficult to estimate, but if we never search the chance of success is zero."

Independently, F. Drake, a young radioastronomer in the then embryonic staff of the National Radio Astronomy Observatory (NRAO) at Green Bank, West Virginia, was planning to undertake such a search with the 85-ft Tatel radiotelescope, the only one then existing at Green Bank. The search was undertaken in the spring of 1960, logging 200 h of observations of two Sun-like nearby stars, Epsilon Eridani (10.7 light yr) and Tau Ceti (11.9 light yr). He used a single-channel receiver and a new parametric amplifier, the best then available, which allowed him to reduce the system temperature from 1,500 K to ~350 K. The project was named OZMA after the Princess of the mythical kingdom of Oz. Drake⁵ reports the excitement of a false alarm they experienced when they first tuned their antenna to Epsilon Eridani. Such heart-stoppers have not been uncommon in projects associated with the search for extraterrestrial intelligence (SETI).

After a rather slow start in the 1960s (only three searches in the first 7 yr with less than 300 h of observations), SETI began to gain momentum in the 1970s, thanks to a meeting at the Byurakan Observatory⁶ in Soviet Armenia which was co-sponsored by the National Academies of both the Soviet Union and the United States, and a series of design studies⁷, workshops^{8,9} and conferences¹⁰, organized by the National Aeronautics and Space Administration (NASA) at its Ames Research Center. Finally in the 1980s it became established as an active branch of both the life sciences (exobiology) and of astronomy (bioastronomy). SETI was also included in the recommended astronomy projects for the 1980s by a special committee of the US National Academy of Sciences¹¹ and gained the endorsement of the International Astronomical Union (IAU), which at its 1982 triennial General Assembly established a new Commission¹² on this subject (IAU Commission 51—Search for Extraterrestrial Life). I was elected president, and N. Kardashev of the Soviet Union and F. Drake of the United States vice-presidents, all for the period 1982–85. The new commission grew rapidly to about 270 members and in 18–21 June 1984 organized in Boston the first IAU Symposium¹³ of this new branch of astronomy, which is rapidly becoming known as bioastronomy. During this symposium we celebrated 25 years since the historic paper by Cocconi and Morrison, while in the

spring of 1985 we celebrated the 25th anniversary of Project OZMA with a special meeting at NRAO in Green Bank which was attended by Drake and many of the pioneers and current workers in this field.

Search projects

In these 25 yr there have been about 50 search projects, most of them at radio frequencies but a few also in the optical and in the infrared. J. Tarter¹⁴ maintains the only active file of all these projects, which I have used as the basis for most of the descriptions that follow, augmented by personal information and data from papers by the experimenters included in ref. 13. So far we have amassed close to 120,000 h of observations using facilities in seven countries (United States, Soviet Union, Australia, Canada, France, Germany and Holland). Japan¹⁵ is also preparing to join the group with its new 45-m Nobeyama millimetre radiotelescope which is equipped with two acousto-optical spectrometers, one with 16,384 channels and a 2-GHz total bandwidth, and the other with 8,192 channels and a 160-MHz total bandwidth. They also have a 4,096 Fourier transform spectrometer with a frequency resolution of 150 Hz–10 kHz.

Almost all of the radio searches up to now have been conducted at selected 'magic frequencies', because from knowledge of science these radio lines would be known both to the transmitting and to the listening civilizations. This idea was advanced by Cocconi and Morrison⁴ who in essence said that if indeed 'they' want to make contact with us, 'they' will make it as easy as possible by transmitting at a frequency that is universally known. At the time, the 21-cm hydrogen line was the only one we knew, and though we now know radio lines of more than 65 atoms, molecules and radicals, the hydrogen line (*primus inter pares*) continues to dominate the searches. Unfortunately this reasoning has not paid off, but there was nothing else we could have done with the technology then available. In the next generation of searches, however, we plan to use multichannel spectrum analysers (MCSAs) with many millions of channels to search over a wide frequency range, rather than focusing only on certain magic frequencies.

Below we review the different searches that have been undertaken in the past 7 yr, or that are in progress. Following Tarter¹⁴, we will divide them into three general categories: Directed, Shared or Parasitic, and Dedicated, which are differentiated by the use they make of their observing facilities. For a description of searches before 1978 see ref. 16.

Directed searches

These searches use a major observatory for a specific SETI project of relatively short duration. Thus, in 1978, Sullivan *et al.*¹⁷ of the University of Washington proposed that an alternative search strategy would be to eavesdrop on nearby stars for radio signals leaking unintentionally into space from other advanced civilizations. As a test, Sullivan and Knowles¹⁸ used the large Arecibo antenna to observe the radio leakage of the

Earth in the 150–500-MHz range from its reflections from the Moon. Strong television stations and powerful military radars are the most prominent sources. As an example, the space surveillance radar of the US Navy in Archer City, Texas, which operates at 217 MHz emitting pulses of 1.4×10^{10} W into a bandwidth of only 0.1 Hz, could have been detected by a civilization with an Arecibo technology to a distance of 20 light yr. Knowles¹⁹ and Sullivan also did a limited eavesdropping search on a few nearby stars. The data were obtained with the Arecibo radiotelescope and the Mark I VLBI (very-long-baseline interferometry) system. They were analyzed for ultra-narrowband signals over a wide frequency range using a computer to create large Fourier transforms from tape-recorded one-bit time histories of the telescope voltage output. This technique, 'one-bit spectral analysis', is no substitute for a large MCSA, but can be used in parallel utilizing otherwise idle computer time. Although this test-run found no signals, it showed that eavesdropping on nearby stars is an alternative search technique that must be explored further.

Other directed searches in the United States include those of Tarter, Clark, Cuzzi, Duquet and Lesyna who used the Arecibo radiotelescope to observe 210 solar-type stars in a 4-MHz band around the magic frequencies of both HI and OH, and who also used the one-bit tape-recording technology and a CDC 7,600 computer to obtain a spectral resolution of 5.5 Hz. Using the 14-m millimetre radiotelescope of the University of Massachusetts, Lord and O'Dea looked for powerful beacons at the 115-GHz (2.6-mm) line of CO along the north rotational axis of the Galaxy, a potentially 'magic location' for beacons operated by supercivilizations.

On the other side of the Atlantic, F. Biraud of the Observatory of Meudon and J. Tarter of Berkeley and NASA-Ames have been using the large (40 × 240 m) radiotelescope at Nançay, France, since 1981, and an eight-level 1,024-channel autocorrelator with a resolution of 50 Hz per channel to observe 300 solar-type stars at four OH frequencies. This project is complementing previous work done with the Arecibo and the 300-ft NRAO radiotelescopes, where low declination sources had not been observed. In Holland, Shostak of the Kapteyn Astronomical Institute and Tarter used in Project SIGNAL the Westerbork array to look with high angular resolution (to screen out noise) for a pulsed beacon, with a repetition rate between 40 s and 1 h, at the centre of the Galaxy, a magic location for a supercivilization.

P. Horowitz of Harvard, while on sabbatical at Stanford, built together with Teague, Linscott, Chen and Backus, an impressively compact MCSA, which they named 'Suitcase SETI'. It had 65,536 (2^{16}) channels for each of the two circular polarizations and a resolution of 0.03 Hz per channel. They used it in Arecibo to observe 250 solar-type stars at the hydrogen line and at twice the hydrogen frequency. They were looking for an ultra-narrowband signal, which is the most economic means for interstellar communications. The Drake-Helou spreading²⁰ due to multiple scattering from inhomogeneities of the interstellar medium, limits the narrowness of the signal to a bandwidth of 0.01–0.1 Hz. This initial experiment later led to Project SENTINEL. Another interesting search was undertaken in 1983 by R. Freitas and F. Valdez who used the Hat Creek radio observatory to conduct a search at the 1,516-MHz line of tritium. Since tritium has a half-life of only 12.5 yr, if found in the vicinity of normal stars it could only have an artificial origin, possibly the by-product of a huge nuclear fusion plant. The same workers had earlier used the 30-inch telescope of the University of California and the 24-inch telescope of the Kitt Peak National Observatory (KPNO) to conduct an optical search for artefacts and probes placed in our Solar System by extrasolar civilizations. They looked at the magic locations of the L4 and L5 Lagrange points of the Earth–Moon and Earth–Sun systems, which are the most stable regions in space to observe the Earth.

Other non-radio search projects include those of V. Shvartsman and his colleagues of the Special Astrophysical Observatory of the Soviet Union, who used the 6-m optical telescope at

Zelenchuskaya, USSR, and a device called MANIA which can detect very short pulses (3×10^{-7} – 3×10^2 s) from optical lasers, to look at 21 stellar objects with unusual spectral properties, hypothesizing that they may be examples of supercivilizations that could be using laser signals for interstellar communications. In a related project, a laser heterodyne receiver operating at 30 THz (10 μ m) is now being built by H. Betz of the University of California-Berkeley that will have 1,000 channels each 1 MHz wide, to undertake an infrared search for extraterrestrial laser signals which in certain circumstances might be used for interstellar communications. Finally, I²¹ am preparing to undertake a search for large artificial objects (such as space colonies and materials processing plants) in our Solar System and especially in the asteroid belt, which is an ideal source of raw materials, to test the theory of galactic colonization, that is, the possibility that the entire Galaxy might already have been colonized by more advanced civilizations. I will be using the Infrared Astronomy Satellite (IRAS) data bank of infrared sources in our Solar System, which is now being prepared at the Jet Propulsion Laboratory by the IRAS Asteroid Workshop²², to look for objects with unusual infrared signatures in our Solar System.

Shared or parasitic searches

These searches either re-analyse for ETI signals old, archived data that had been obtained for other purposes, or they share in a parasitic or piggyback fashion the data being obtained by a radiotelescope for an unrelated project and process them for ETI signals in real time. Thus by re-analysing many of the 21-cm 'noisy' sky maps that had been obtained with the Westerbork array and had been kept by the Dutch astronomers, F. Israel of the Netherlands working first with DeRuiter and then with Tarter, looked for strong sources in positions that coincided with those of known stars. It is hoped that a similar process will be used in the future with maps obtained with the VLA (Very Large Array). Meanwhile, Cohen *et al.*²³ re-analysed their own surveys of globular clusters, looking for narrowband signals at frequencies associated with OH and H₂O masers that might be tapped by supercivilizations to produce strong signals in certain directions. M. Damashek is re-analysing for ETI signals 700 h of data that he had obtained with the NRAO 300-ft antenna during an extensive pulsar search at 390 MHz over most of the sky.

In 1980, S. Bowyer and D. Werthimer of the University of California-Berkeley and their collaborators^{24,25}, built an automated 100-channel spectrum analyser to siphon through it, from the intermediate frequency (IF) stage, data obtained by a telescope for an unrelated project. This parasitic or piggyback SETI device was named SERENDIP and was used with the Hat Creek and Golstone antennas. It was recently upgraded to SERENDIP II, which has a 65,536-channel fast Fourier processor with a resolution of 2 Hz per channel. It will be used to search for narrowband peaks above a 6σ threshold over the entire 30-MHz IF band of the radiotelescope searching through increments of 100 kHz in 10 s or less. Data of unidentified peaks are recorded for further investigation. The system will operate unattended on a 24-h basis with practically any radiotelescope.

Many other parasitic searches have been carried out around the world. Thus while searching for impulsive radio events at 5 GHz with the 64-m Parkes radiotelescope in Australia, T. Cole and R. Ekers also checked a few G and K main-sequence stars for ETI signals of short duration. In a 4-day run they detected only one such signal in the direction of 82-Eridani which lasted only 1 ms. Efforts to re-acquire it were unsuccessful. During a routine search for pulsars with the large 100-m Bonn (Effelsberg) antenna, R. Wielebinski and J. Seiradakis of the Max Planck Institute for Radio Astronomy, looked for ETI pulsed signals at the hydrogen line with repetition periods between 0.3 and 1.5 s. And during a study of the galactic magnetic field with the 46-m Algonquin radiotelescope of Canada at 10.6 GHz (2.8 cm), Vallee²⁶ and Simard-Normandin of the Herzberg Institute also looked for highly (>70%) linearly polarized signals, which

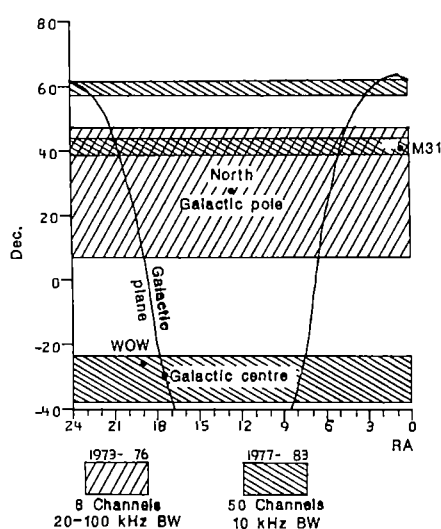


Fig. 1 The regions of the sky that have been surveyed so far by the Ohio SETI Program³².

could be strongly suggestive of an artificial origin, near the centre of the Galaxy where a supercivilization could be operating an intergalactic relay station. Taking advantage of the fact that the 64-m antenna of NASA's Deep Space Station in Tidbinbilla, Australia, was stowed for several months for structural repairs, Gulkis, Kuiper, Olsen, Jauncey and Peters of the Jet Propulsion Laboratory used it for a SETI sky survey at 8 and 22 GHz with a 256-channel spectrum analyser. The elevation of the telescope was occasionally changed to scan a different band of the sky passing over it. Finally, the 1.5-m Mt Lemon optical telescope was used by F. Witteborn of NASA-Ames to further study 20 stars that in a routine infrared study had been found to be faint in the optical for their spectral type, but strong in the infrared. This made them potential candidates for Dyson Spheres, that is, the products of astroengineering activity by supercivilizations which could use all the planetary matter of their solar system to build a huge shell around their central star to tap a large fraction of its energy. Nothing was discovered that could not be explained by natural processes.

Turning to the Soviet Union, Slysh²⁷ and Kardashev²⁸ have also been looking in recent years for Dyson Spheres and other superstructures of astroengineering activity, searching primarily through the IRAS data for stellar sources with an unusual infrared signature. An all-sky search for microwave background fluctuations is under consideration by the Soviet workers to back up the IRAS data. Unfortunately during the past seven years there have been no radio searches in the Soviet Union, where there was considerable SETI, or CETI (Communication with ETI) as Soviet workers prefer to call it, activity in the 1960s and 1970s^{29,30} under the leadership of V. S. Troitskii and N. S. Kardashev. In the Proceedings of the Montreal meeting in 1979³¹ and at the SETI '81 meeting in Tallinn Estonia, Soviet workers had reported that they were preparing to start building in Gorki under the direction of Troitskii an array of ~100 1-m parabolic antennas (Project OBZOR) for SETI work at 21 cm. During my visit to Moscow in August 1984, I was told that this project had been abandoned, primarily because of the long illness of Troitskii—a pioneer and an international authority in this field. The emphasis in the Soviet Union is now geared towards the detection of astroengineering structures by supercivilizations, but I feel that under the leadership of Kardashev and Slysh, they will regain their high-ranking position in the radio searches, especially with the use for SETI of the new 65-mm Samarkand millimetre radiotelescope now approaching completion.

Dedicated searches

Dedicated searches are performed by radio observatories that have become SETI dedicated facilities and conduct searches on a continuous basis. There are two such projects now in operation,

which account for ~80% of the observing hours accumulated thus far. The oldest of the two is the Ohio SETI Program, having been in operation since 1973 under the direction of J. Kraus and R. Dixon³². It uses the meridian-transit radiotelescope of the Ohio State University which has a collecting area of 2,200 m², equivalent to a parabolic dish 53 m (175 ft) in diameter, and a beam pattern at 21 cm of 8 arc min in RA and 40 arc min in dec. The search is conducted at the hydrogen line, Doppler shifted in the rest frame of the galactic centre, with the help of a 50-channel filter bank with a resolution of 10 kHz per channel. During the first 3 yr (1973–76), however, the system was less sophisticated and operated with only eight channels. Figure 1 shows the portions of the accessible sky (–36 to +63 in declination) that have been covered thus far by the old and the new system. The sky band 40–43 dec was chosen because it includes the Andromeda Galaxy (M31), our large neighbouring galaxy.

The 'WOW' signal of the Ohio SETI Program (Fig. 1) was recorded in 1977 at –27 dec. It earned its name from the word the telescope operator penned next to this peak on the computer printout of the telescope, and in spite of repeated efforts by the Ohio group and others it has never been re-acquired. The data obtained are archived and analysed for statistical effects with respect to celestial coordinates. Several improvements are being planned for the future, including expanding the frequency range to cover the entire 'water hole' (1,400–1,750 MHz) which is bracketed by the hydrogen and the OH lines, that together form water. The Ohio SETI Program has been sustained with modest support from NASA, the tireless efforts of Kraus and Dixon, and the unselfish dedication of many enthusiastic volunteers.

Horowitz³³ of Harvard University and his collaborators have been operating since March 1983 the other dedicated facility at the Oak Ridge Harvard-Smithsonian Observatory near Boston. It is called Project SENTINEL and is supported by the Planetary Society, a private organization headed by C. Sagan and B. Murray. It is an outgrowth of project Suitcase SETI mentioned earlier, and uses the same two 65,536-channel spectrum analysers, with a resolution of 0.03 Hz per channel, with the 84-ft antenna of the Oak Ridge Observatory. With the telescope set at a particular declination, the search sweeps a 0.5° band around the sky, covering the entire available sky (about 80% of total) in ~1 yr. During their 2 yr of operation (1983–85) they have covered all of the accessible sky, first at the hydrogen line at 1,420.40575 MHz and then at one of the four OH lines at 1,667.3590 MHz. A potential source stays in the antenna beam for about 2.5 min. The system searches automatically for large peaks in any of its channels and archives anything suspicious. Because of the expected swept-Doppler signature produced by the Earth's rotation, the system is very good in rejecting radio interference, though they too had two false alarms of almost 50σ when they got the Sun into their beam.

The problem with this search is its very narrow (2 kHz) total bandwidth around the hydrogen line. This necessitates a continuous correction for all the Doppler shifts due to the motions of the Earth relative to the direction of the antenna, and assumes that the transmitting civilization will also correct for all Doppler shifts, including the relative motion of our two stars (the radial component of the peculiar motion) which can be of the order of 100 kHz, that is 50 times larger than the 2-kHz total bandwidth of the system. It also requires that they beam their signals specifically to our Solar System. To overcome these restrictions, Horowitz and his colleagues³⁴ have built a brand new MCSA with 8.4×10^6 channels and a frequency resolution of 0.05 Hz per channel, which is giving them a total bandwidth of 420 kHz, enough to account for practically all of the Doppler effects. In the 2.5 min that a source stays in their antenna beam the system will make six and possibly eight measurements checking the left-hand and the right-hand circular polarization at three or four distinct frequencies of the same line resulting from Doppler effects in three or four different rest frames (the local standard of rest of our Solar System and possibly their heliocentric frame, the reference frame of the galactic centre, and the cosmic black-body rest frame in which the 3 K background radiation is

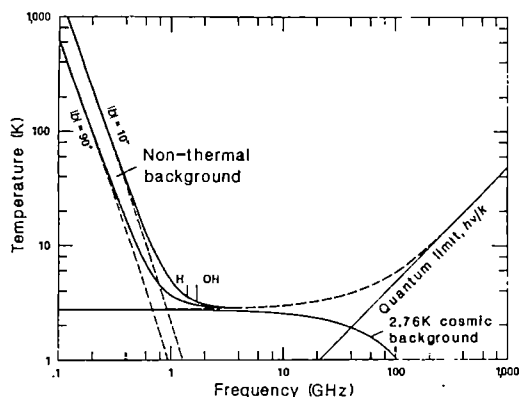


Fig. 2 The microwave window of interstellar space³⁸.

isotropic). This new system, which has been named Project META, went into operation on September 29, 1985.

Technological progress and NASA's Program

The search for ETI radio signals is a multidimensional problem which because of its complexity is often called the 'cosmic haystack'. The dimensions of the search space include: location of sources; transmitting frequency; signal strength; bandwidth; polarization; signal modulation; and on-off periods. Initially investigators in the United States had hoped that ETI would be transmitting at the hydrogen line to make contact easy. But extensive, although not exhaustive, searches by many investigators at hydrogen and a few other magic frequencies have not produced any positive results, which indicates that the search is not going to be an easy task. With the improvement in our technology, NASA is now preparing to embark on the next generation of radio searches, that is a systematic search over a wide frequency range (rather than the old magic frequencies) in the microwave window (1–10 GHz) of the Earth. This is the free-space microwave window seen on Fig. 2, cut off for ground observations around 10 GHz by the water vapour in the Earth's atmosphere. This frequency range is relatively free of natural radio noise, and is therefore best suited for interstellar communications. Embedded deep in the microwave window is the 'water hole' (1.4–1.7 GHz), probably the most attractive frequency range for interstellar radio communications. A point of great concern, however, is the rapidly growing use of this frequency range for other purposes, which are bound to interfere with future radio searches.

It is generally believed that radio signals will be narrowband to reduce peak power. To have a good signal-to-noise ratio we need receivers with very narrow bandwidths, and since we need to explore a wide frequency range we need a large number of narrowband channels. This function is fulfilled by a MCSA³⁵, an electronic high-resolution radio spectrometer, which in the past 25 yr has evolved from a few channels to 65,000 channels and we are soon to have MCSAs with 8×10^6 channels and possibly more. We have already mentioned the ultra-narrowband MCSA constructed by Horowitz for his Project META. NASA is also planning an 8.25×10^6 -channel MCSA, but with a much wider total bandwidth, which is now being developed by a group at Stanford University headed by Peterson³⁶ and Linscott. It will consist of two cascaded banks of digital filters followed by multiplexed microprocessors executing digital Fourier transforms. The complete MCSA will have 112 units, each with 73,728 channels for a total of 8,257,536 channels. The highest frequency resolution will be 1 Hz per channel, giving a total bandwidth of about 8 MHz. It will also be able to analyse simultaneously the incoming 8-MHz frequency band into channels of 32, 1,024 and 73,728 Hz. In all cases, it will examine both the left-hand and the right-hand circular polarizations.

The MCSA performs a complex Fourier transform that yields the real and imaginary amplitude of the signal, which are then

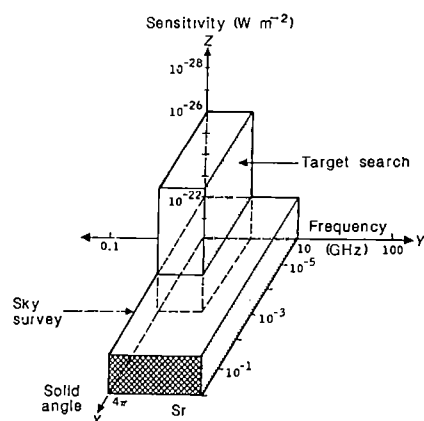


Fig. 3 The two 'volumes' of the SETI Search Space, that will be searched by the Sky survey and the Targeted search, the two complementary components of the NASA SETI Program⁴⁰.

squared and added to give the actual power for each bin (channel). If it exceeds a predetermined threshold, chosen by the desired signal-to-noise ratio, the signal is flagged for further tests. Sophisticated signal detection algorithms^{35–37} are now being developed by NASA. Considerable effort is being made to achieve online processing of the data, which is not an easy task given the huge volume of incoming data with an 8×10^6 -channel MCSA. Emphasis is also placed on the ability to detect pulsed signals and signals with a Doppler frequency drift³⁷. The 8×10^6 channel MCSA could be ready by 1988, but more realistically the whole system, including all the signal processing instrumentation, will probably become operational in the early 1990s. A prototype unit with 73,728 channels, together with several of the new signal recognition algorithms, is now undergoing tests at the Goldstone antenna. In a recent test, they were able to pick up the very weak signal (1 W) beamed by Pioneer 10 towards the Earth from a distance of ~35 AU ($\sim 5,250 \times 10^6$ km). They were also able to see clearly the frequency drifts imposed on this monochromatic signal by the rotation of the Earth and the relative motion of the Earth and the spacecraft.

The NASA SETI Program³⁸, which has been in the study and development stage for the past 15 yr, is now headed by B. Oliver, one of the pioneers of SETI⁷, and includes about 15 well-known scientists at the three California institutions (NASA-Ames, Jet Propulsion Laboratory, and Stanford University) that share responsibility for this programme. The NASA SETI Program will be a bimodal search consisting of two components: the Targeted search³⁹, which will emphasize sensitivity to weak signals by concentrating only on a number of discrete sources, and the Sky survey^{40–42}, which will emphasize sky coverage by scanning the entire sky. Figure 3 shows the relative volumes of the search space that will be investigated by these two complementary searches.

The Targeted search will focus on 800–1,000 specific targets, including the 773 F, G and K Sun-like stars up to a distance of 25 pc (81.5 light yr) included in the Royal Greenwich Observatory Catalogue, and a variety of other targets including stars with peculiar spectra and galaxies. It will have a peak spectral resolution of 1 Hz and will concentrate in the frequency range surrounding the water hole. The sensitivity (Φ) of radio observations, usually given in W m^{-2} , is related to the demanded signal-to-noise ratio (a), the system temperature (T), the collecting area of the antenna (A) which is proportional to the square of the diameter (D) of the antenna, the resolution bandwidth (b), and the integration time (τ), by the expression:

$$\Phi = a \frac{kT}{A} \left(\frac{b}{\tau} \right)^{1/2} \quad (1)$$

Since for given Φ , T and b , τ is proportional to D^4 , to achieve the highest possible sensitivity in a reasonable time, the targeted

search will use the largest possible antennas including the 305-m Arecibo, the 53-m (equivalent) Ohio State, the 64-m Goldstone (California) and Tidbinbilla (Australia) antennas of NASA's Deep Space Network, the 91-m NRAO, and possibly the 100-m Bonn (Germany) antenna. It is interesting to note that when the 1,000-ft Arecibo antenna with a system temperature of 35 K is compared with Drake's 85-ft NRAO antenna with a 350 K system temperature, Arecibo would be able to do the 200 h of Project OZMA in just a fraction of a second.

An approximate time calculation for the Targeted search yields 1,000 targets \times 1,000 s per target per 8-MHz frequency band \times 100 frequency bands to cover the range 1.2–2.0 GHz around the water hole $= 10^8$ s $=$ 3.3 yr, which, including losses of time for moving the antenna from target to target, antenna maintenance, transportation of the MCSA from station to station, and so on, amounts to \sim 5 yr of telescope availability. The Sky survey will use several of the smaller, 34-m radio-telescopes in NASA's Deep Space Network. It will have a lower spectral resolution of 32 Hz per channel, but will cover a wider frequency range (1–10 GHz). It will survey the entire sky (4π sr) with a half-power beamwidth (HPBW) of $\sim \lambda/D$ and therefore will, in essence, examine $4\pi/(\lambda/D)^2 \sim 10^6$ locations. Spending from 0.3 to 3 s per location (to scan through a HPBW) and per frequency band of about 250 MHz, and needing \sim 36 such bands to cover the 1–10 GHz range, yields again a total of $\sim 10^8$ s, that is also \sim 5 yr of telescope availability. Thus the entire NASA SETI Program (Targeted search and Sky survey) will require \sim 10 yr telescope availability. Using 5–10 different radiotelescopes at \sim 10–20% of their time, and assuming the availability of several MCSA units, the whole programme will take optimistically \sim 5 yr, but more realistically probably close to 10 yr. Hence assuming a starting date around 1990, we can expect it to be completed around the year 2000, having enhanced immensely the volume of the search space that we will have explored. It is also possible that a high spectral resolution search, such as this, over the entire sky and over a wide frequency range, might produce also some unexpected astronomical discoveries.

Rationale behind the search strategy

Sparked off by a critical paper by Hart⁴³ in 1975, there were many heated debates⁴⁴ and counter-arguments⁴⁵ in the late 1970s and early 1980s on the most advisable search strategy and even on the advisability of undertaking any searches at all. The key points to these debates were: (1) the possibility of interstellar travel and of galactic colonization, and (2) the lack of any scientifically verifiable evidence of visits to Earth by extraterrestrials, which is often referred to as the 'Fermi Paradox' or the 'Great Silence'⁴⁶. After many debates, we began to realize that none of us can claim to know how civilizations far more advanced than ours are likely to behave and act. The conclusion, therefore, which was stated by several participants^{46–50} at the recent IAU Symposium¹³ can be summarized as follows: debates, useful as they might be to sharpen our understanding of the issues, will never answer our questions. The answers can come only through scientific searches, which must be pursued vigorously. The search strategy, however, ought to be broad and flexible enough to allow, besides the mainstream searches such as the NASA SETI Program, the parallel experimental testing of different other theoretical possibilities. With such a broader search strategy we are likely to keep many more good people active in this young field, and at the same time increase our chances of success by not neglecting any of the possible alternatives.

From a comparison of Project OZMA and Arecibo with an MCSA, it is clear that we have made colossal technological progress in less than three decades. A question often asked is why we do not postpone our searches until our technology becomes more effective. I believe the answer has two parts. The first is that we do not know in advance the level of technology that would be sufficient. It would have been a grave mistake to have asked Wilbur and Orville Wright to wait for the discovery of the jet engine before trying to fly. They succeeded with far

less, and on 17 December 1903 they opened the doors of the new field of aviation. I am sure that even if Drake could have known that 25 yr later Horowitz would have had an 8×10^6 MCSA for a search around the hydrogen line, he still would have gone ahead with his Project OZMA, and rightfully so. The second reason is that technology is like a ladder that we must climb one rung at a time, starting from the lowest ones. But as we start climbing, the horizons broaden and many new technological developments materialize, such as the jet engine in aviation and the 8×10^6 MCSA in SETI, with benefits also for many other fields.

After millennia of thinking and philosophizing about the 'plurality of worlds', we have finally entered the experimental era. We have already used space probes to search for primitive life in our Solar System, and we are now searching with radio, optical and infrared telescopes for other advanced civilizations in the Galaxy. It is a special privilege to live in the era that tries to answer experimentally profound old questions about the prevalence of life, and especially of life with intelligence, in the Universe. With the NASA SETI Program as the central force, and the many other parallel special searches now in progress or planned for the future, we can expect that in the next 10–20 years we will know much more about the presence of other advanced civilizations in our Galaxy. If we were to find them, this would be the greatest discovery of all time. But even if after concerted efforts we were to conclude that we must be one of very few if not the only advanced civilization in the Galaxy, this too would be an important discovery, because knowing how rare our civilization is among the hundreds of billions of stars in our Galaxy would hopefully make us realize how cosmically important it is to preserve it.

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ARTICLES

Intensive measurements of turbulence and shear in the equatorial undercurrent

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Average profiles of turbulent dissipation rates provide tests of parameterizations used in numerical models. A strong diurnal mixing cycle is found in the well-stratified high-shear zone above the velocity maximum, and thermohaline intrusions are shown to be important mixing agents below the velocity maximum.

INTENSE and persistent mixing occurs in the equatorial undercurrents of the Atlantic and the Pacific oceans. Determining and parameterizing the average turbulent fluxes is necessary because these fluxes may provide the primary dynamical balance to the pressure gradient driving the undercurrent. Turbulent transport in the undercurrent is particularly important in the central Pacific, where it is a major factor controlling the tongue of cold water extending westward from South America¹. Temperature changes of the cold tongue affect the air-sea interactions that lead to anomalous weather conditions over North America. Therefore, improving the measurement and parameterization of the vertical turbulent fluxes in the undercurrent is a major goal of the Tropic Heat Program established to study the cold tongue¹. The undercurrent also provides an excellent natural laboratory for studying stratified turbulence.

Although the amount of previous turbulence data from the undercurrent is small, microstructure profiles taken at various locations and times, with different instruments, show similar patterns and intensities^{2–5}. Earlier measurements with towed bodies report levels at the core of the undercurrent that are several decades higher than those observed with profilers^{6,7}, leading some to conclude that the towed data are badly contaminated by vibration and sensor difficulties^{2,5}. Others maintain that large intermittence at the velocity maximum produces the disparity⁸.

Between 25 and 30 November 1984, during the Tropic Heat field measurements, continuous profiling of turbulence was done in the upper 150 m at 0° N, 139°50' W with the advanced microstructure profiler (AMP)⁹. In 4.5 days, 385 drops were obtained, providing enough data to form accurate ensemble average profiles and to examine short-term fluctuations. Also, eight shear measurements were obtained with the expendable current profiler (XCP)¹⁰.

Background conditions

The average stratification and shear are typical of conditions near 0° N, 140° W. The mean stability frequency, \bar{N} , is low near the surface but increases rapidly, reaching a maximum of 0.02 s^{-1} near a pressure (p) of 1.0 MPa (corresponding to a depth of 100 m). Close to the surface the stratification is controlled by temperature, but salinity is important below 0.65 MPa. Because the XCP measures relative velocities, the average speed between 5.0 and 5.5 MPa is set to zero, in general agreement with mooring observations. Useful signals from the XCP begin at 0.3 MPa;

the transport at shallower depths is to the west, as observed from the ship drift. The zonal flow below 0.3 MPa is eastward (zonal velocity $\bar{U} > 0$ in Fig. 1), reaching 1.6 m s^{-1} near 1.05 MPa, which is close to the depth of the salinity maximum. Owing to the proximity of the magnetic equator the north component of the XCP signal is not useful. Nevertheless, because of the dominance of the eastward flow, good estimates of shear are obtained from $\partial \bar{U} / \partial z$ (where z is the depth). Values are calculated from the slopes of linear fits to the raw velocity components. The interval of each fit is 0.2 MPa, and a fit is done every 0.05 MPa. Shear decreases with depth and has a local minimum at the velocity maximum. Because this occurs close to \bar{N}_{max} , the Richardson number, $Ri \equiv \bar{N}^2 (\partial \bar{U} / \partial z)^{-2}$, has a sharp maximum. Above 0.5 MPa, $Ri < 0.25$; between 0.5 and 0.9 MPa, it is about 0.35 (Fig. 2). Based on fluctuations in the eight shear profiles used to form the average, we estimate typical confidence limits to be (0.0–0.15) for $Ri = 0.07$ and (0.2–0.5) for $Ri = 0.3$. Vertical smearing, due to displacements by internal waves and the internal tide, makes the velocity maximum appear thicker and reduces the maximum value. $Ri < Ri_{\text{cr}} \equiv 0.25$ is a necessary but not sufficient condition for instability of laminar flows¹¹.

Average mixing rates above velocity maximum

Centimetre-scale gradients of horizontal velocity and temperature measured with AMP are used to compute the variances of the gradients over successive 0.005 MPa (0.5-m) sections. Scaling yields $\epsilon = 7.5 \nu \langle (\partial U' / \partial z)^2 \rangle$ and $\chi = 6 \kappa_T \langle (\partial T' / \partial z)^2 \rangle$. ϵ is the rate of viscous dissipation of turbulent kinetic energy, and χ is the rate of diffusive smoothing of turbulent temperature fluctuations. (ν is the kinematic viscosity and κ_T is the thermal diffusivity.) In both cases, isotropy of the microscale gradients is assumed.

Because of the large number of records taken in a short time, average profiles of ϵ and χ can be formed using all data in each 0.005 MPa pressure bin (Fig. 3). Comparison with laboratory results provides a measure of the relative intensity of $\bar{\epsilon}$. Studies of decaying grid turbulence in unsheared flows¹² show that when $\epsilon > \epsilon_{\text{tr}} = 24.5 \nu N^2$, the turbulence will be sufficiently intense to produce a negative buoyancy flux, that is, $J_b \equiv -g \rho^{-1} w' \rho' < 0$ where g is the gravitational acceleration, ρ' is the density and w' is the vertical velocity. A negative buoyancy flux corresponds to an upward mass flux, which increases the mean potential energy. Preliminary results from sheared flows¹³ show that the

Fig. 1 Average profiles of the stratification and velocity fields; θ is potential temperature, s is salinity (in concentration units), σ_θ is potential density, p is pressure, and U is the zonal velocity (positive east). These plots are formed from averages of 385 AMP profiles and eight XCP profiles. Owing to vertical displacement by internal waves and the internal tide, averaging smooths small-scale structures in these profiles. Both the depth of the velocity maximum, 1.1 MPa (110 m), and its association with the salinity maximum are typical near 0° N, 140° W. The stability frequency, N , is computed from the average temperature and salinity profiles and peaks slightly above the velocity maximum, producing a sharp maximum in the Richardson number, Ri .

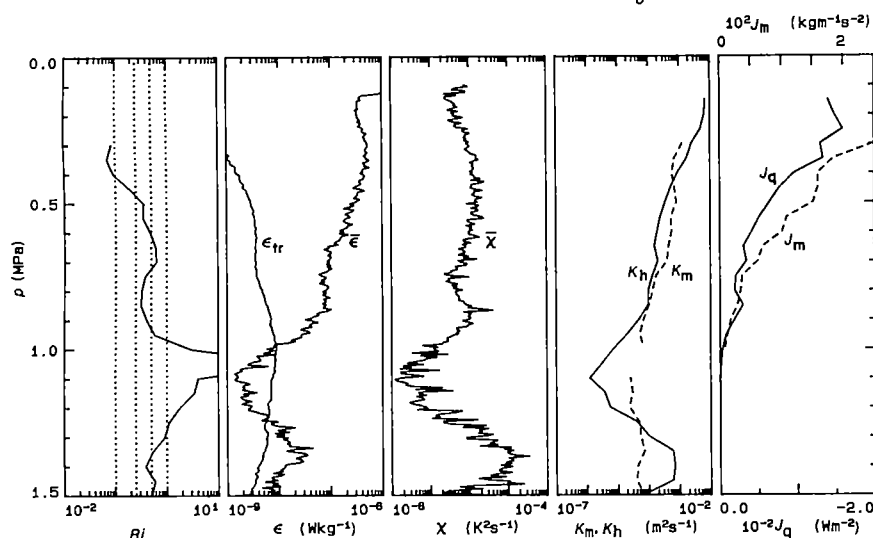
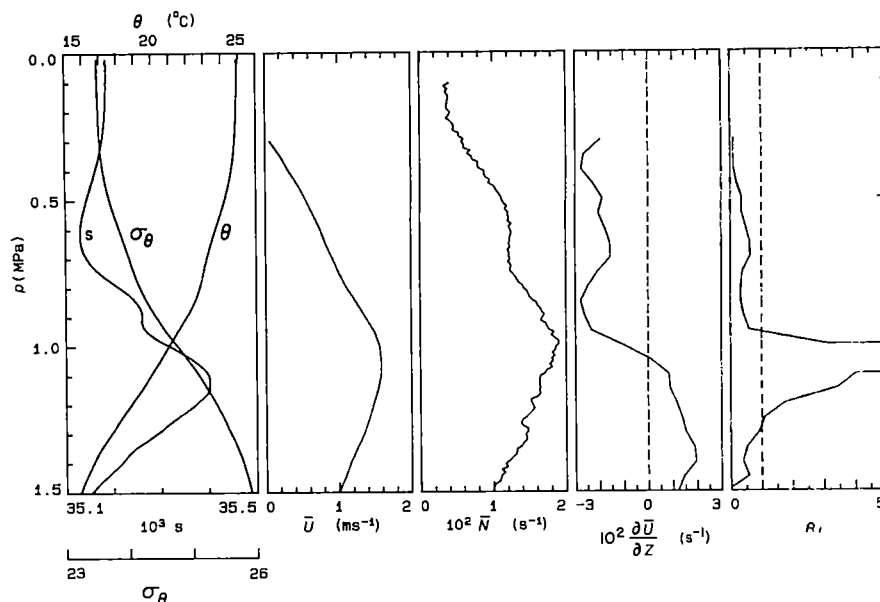


Fig. 2 The average Richardson number, Ri , and dissipation rates. Where $Ri < 1$, $\bar{\epsilon}(p) > \epsilon_{tr}$, the threshold for active turbulence. $\bar{\chi}(p)$ follows a similar pattern, modified by the local temperature gradient. The turbulent eddy coefficients for heat, K_h , and momentum, K_m (dashed line), are approximately equal between 0.3 and 0.9 MPa. The corresponding turbulent fluxes, J_q and J_m (dashed line), follow similar patterns, having large values near 0.3 MPa and decaying to about 0 at the Ri maximum.

same criterion holds when $Ri > 0.25$; when $Ri < 0.25$ the turbulence does not decay.

In the undercurrent, $\bar{\epsilon}$ decreases with depth (Fig. 2). Above 0.4 MPa, $Ri < 0.1$ and $\bar{\epsilon}$ exceeds ϵ_{tr} by at least two decades. This ratio decreases to about 20 between 0.6 and 0.9 MPa, where $1/4 < Ri < 1/2$. It then drops rapidly and crosses 1 where $Ri = 1$. Thus, in most of the high-shear zone above the velocity maximum the average dissipation rates indicate strongly active turbulence, producing a net buoyancy flux.

In steady, homogeneous turbulence, the balance equation for turbulent kinetic energy reduces to local production and dissipation. If the mean shear is strong, the turbulent production can be expressed as a vertical eddy coefficient for momentum, K_m

$$K_m = \frac{\epsilon}{(1 - R_f)(\partial \bar{U}/\partial z)^2} \quad \text{m}^2 \text{s}^{-1} \quad (1)$$

where R_f is the flux Richardson number. Because $R_f \leq 0.15$ this term is a small correction and is ignored. The corresponding vertical momentum flux is

$$J_m = -\rho K_m \frac{\partial \bar{U}}{\partial z} \quad \text{kg m}^{-1} \text{s}^{-2} \quad (2)$$

In studies of the planetary boundary layer of the atmosphere, this procedure is known as the dissipation method and has been verified by comparison with direct flux measurements. If the temperature fluctuations are produced by turbulent overturns against the mean gradient, rather than by lateral advection, a similar balance applies with the T^2 -balance equation, yielding

the turbulent transport coefficient for heat, K_h , and the vertical turbulent heat flux, J_q :

$$K_h = \frac{\chi}{2(\partial \bar{T}/\partial z)^2} \quad \text{m}^2 \text{s}^{-1} \quad (3)$$

$$J_q = -\rho C_p K_h \frac{\partial \bar{T}}{\partial z} \quad \text{W kg}^{-1} \quad (4)$$

This approach, known as the Osborn-Cox method¹⁴, is widely used to obtain turbulent heat fluxes in the thermocline.

Equations (1) and (3) have been used with previous measurements in the undercurrent to develop parameterizations for numerical models^{15,16}. Their use has not been verified in the ocean by comparison with direct flux measurements, but conditions between 0.3 and 0.9 MPa are more appropriate to the dissipation method than any other known section in the ocean, and the present data offer much greater statistical confidence than previously available. Above 0.9 MPa, K_m and K_h are nearly equal, that is, the turbulent Prandtl number is close to one, and they decrease rapidly with depth. At 0.3 MPa, $K_m \approx K_h \approx 2 \times 10^{-3} \text{ m}^2 \text{s}^{-1}$, and at 0.9 MPa, $K_m \approx K_h \approx 6 \times 10^{-5} \text{ m}^2 \text{s}^{-1}$, a decrease by a factor of 25. Over the same interval, J_q drops from 140 to 20 W m^{-2} . (J_q decreases relatively less than K_h because $\partial \bar{T}/\partial z$ also decreases.)

Average mixing rates at velocity maximum

At the velocity maximum, the dissipation rate has a minimum, $\bar{\epsilon} = 2 \times 10^{-9} \text{ W kg}^{-1}$, which is about a quarter of ϵ_{tr} . Because $\epsilon < \epsilon_{tr}$, dissipation-scale fluctuations are less likely to be isotropic

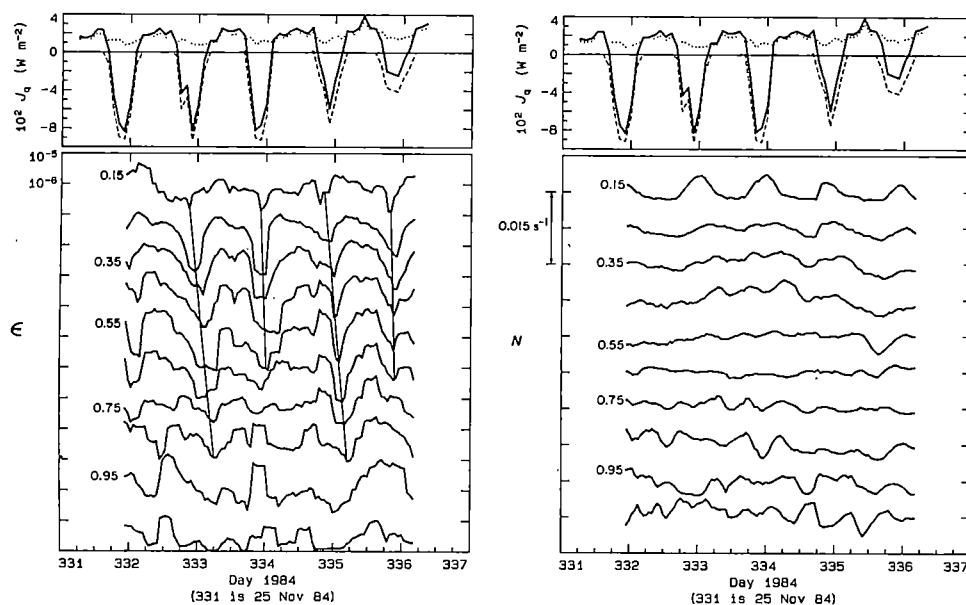


Fig. 3 Five-hour running means of the surface heat flux, of ϵ , and of N , showing a diurnal cycle in ϵ penetrating deep into the thermocline. The surface heat flux (solid line in the top panels) is dominated by the short-wave radiation (dashed), and by the latent heat flux (dotted). The time series for ϵ and N are averages over successive 0.1-MPa intervals; for example, the first set, labelled 0.15, are averages between 0.1 and 0.2 MPa. The curves for pressures >0.15 MPa are successively offset by one decade in ϵ and N , respectively. The diurnal cycle in ϵ remains distinct to 0.65 MPa and has some deeper signatures, unlike the diurnal N cycle, which is weak at 0.25 MPa and absent deeper. The daily minimum in ϵ has a progressive phase lag with depth, as shown by the slanting lines.

than at shallower depths. The maximum effect of anisotropy is to reduce $\bar{\chi}$ by a factor of 3. The effect on $\bar{\epsilon}$ is more difficult to estimate, but should be at least as large. Thus, if the microstructure at the core is anisotropic in the dissipation range, $\bar{\epsilon}$ and $\bar{\chi}$ will be even smaller compared with the levels in the high-shear zone shown in Fig. 2.

To remove the effects of displacements by internal waves and the internal tide, a preliminary statistical analysis was done on all 0.5-m ϵ values between potential density, $\sigma_\theta = 24.6$ and 25.5, which spans the velocity maximum. This yields $\bar{\epsilon} = 4.7 \times 10^{-9} \text{ W kg}^{-1}$ which is only a factor of three higher than an average found in a quiet region of the California Current¹⁷ and is almost three decades lower than the values reported from towed measurements^{6,7}. A probability distribution does not reveal any greater degree of intermittence than in other locations. For example, using the standard deviation of $\ln \epsilon$ as the measure of intermittence⁸, $\sigma_{\ln \epsilon} = 2.4$ compared with $\sigma_{\ln \epsilon} = 2.3$ from the California Current¹⁷. Using this in the maximum-likelihood estimate suggested for intermittent turbulence⁸ yields a value smaller than the arithmetic mean, that is, $\epsilon_{\text{mle}} = 4.2 \times 10^{-9} \text{ W kg}^{-1}$, because the distribution is skewed to low values and has a median of $2.2 \times 10^{-10} \text{ W kg}^{-1}$. Therefore, these data do not reveal unusual intermittence at the velocity maximum, but indicate that the conditions are similar to those in the main thermocline at less energetic locations.

Because the shear passes through zero, conditions are not appropriate for the dissipation method, and K_m is not calculated. The estimate of K_h is not affected, however, because the temperature gradient remains large, and $K_h \approx 1 \times 10^{-6} \text{ m}^2 \text{ s}^{-1}$. This is about 10 times κ_T , the molecular diffusion coefficient, and provides additional evidence that mixing levels at the velocity maximum are weak.

The diurnal cycle

Daily fluctuations, by factors of 10 to 100 in ϵ and χ , exist both in the diurnal mixed layer and in the well-stratified profile below (Fig. 3). The cycle extends to between 0.75 and 0.95 MPa, even though day-to-day variability not related to changes in the surface heat flux is also apparent.

Between 0.1 and 0.3 MPa, both the mixing and the stratification change in phase with the surface heat flux, which is dominated by the short-wave radiation and the latent heat flux. (The sensible heat flux is negligible by comparison, varying between -12 and $+20 \text{ W m}^{-2}$.) At night, the net flux is $\approx 200 \text{ W m}^{-2}$ out of the sea, corresponding to a surface buoyancy flux of $J_b^0 = 3.2 \times 10^{-7} \text{ W kg}^{-1}$, producing a well-mixed layer extending to between 0.2 and 0.3 MPa.

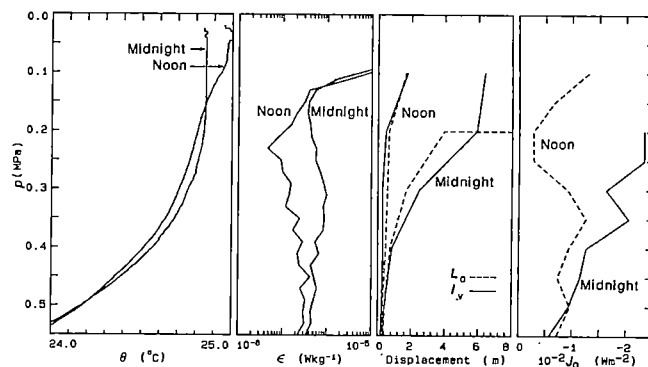
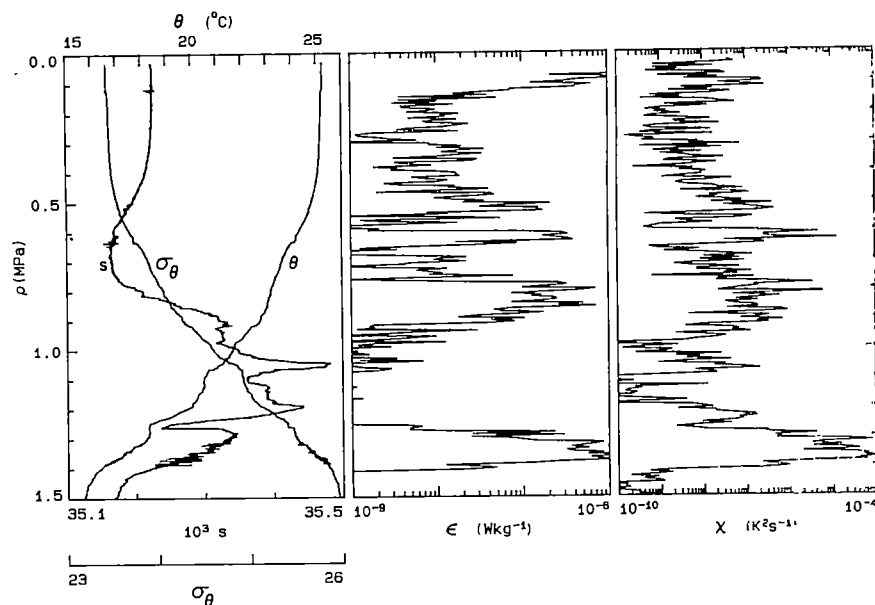


Fig. 4 Comparison of the averages of all profiles within 1 h of local noon and of local midnight. The mixed layer extends to 0.2 MPa at midnight, but is fully restratified by noon. Above 0.1 MPa the mixing rates are dominated by wind forcing, but between 0.15 and 0.65 MPa the diurnal cycle is evident. At noon, the turbulence is most heavily suppressed near 0.25 MPa. The observed vertical displacements, l_v , are independent measures of changes in ϵ and agree well with the Ozmidov scale, L_o , computed from the dissipation rates and stratification. At 0.25 MPa, the turbulent heat flux, J_q , drops from 240 W m^{-2} at midnight to 30 W m^{-2} at noon.

Observations in another convecting mixed layer show $\bar{\epsilon} \approx J_b^0$ within the mixed layer and below the zone affected by the wind¹⁸. In this case, which was in a region with a moderate mean shear, the mixed layer terminated in a sharp density step and the dissipation rates below the entrainment zone decreased several decades. A profile of $\bar{\epsilon}$ for midnight shows a minimum of $4 \times 10^{-7} \text{ W kg}^{-1}$ below the surface zone, followed by an increase to $1 \times 10^{-6} \text{ W kg}^{-1}$ at 0.3 MPa (Fig. 4). Thus, the turbulence produced by convection may account for a substantial fraction of the observed $\bar{\epsilon}$ above 0.3 MPa, but production by the mean shear or wind mixing is also present. The shape of the average midnight temperature profile is similar to individual realizations and shows a smooth transition to the stratified regime. This is very different from the abrupt termination of convecting mixed layers where the deeper water has low dissipation rates¹⁸. We believe this is due to the intense turbulence in the stratified section as well as in the mixed layer. Above 0.3 MPa, $\bar{\epsilon}$ is a minimum at noon, when the profile has maximum stratification. Below 0.3 MPa the diurnal ϵ cycle remains large and is 1.5 decades at 0.65 MPa (Fig. 3), even though there is day-to-day

Fig. 5 An AMP drop showing multiple salinity maxima and minima below 1 MPa, indicating the importance of lateral advection. The deepest of the three intrusions occurs where $Ri < 1$ and has strong mixing between 1.25 and 1.4 MPa, where the profile is diffusively unstable to salt fingering. The pattern of activity is similar to that found in highly sheared intrusions in a warm-core Gulf Stream ring¹⁶. This profile was taken at 20.53 GMT, or 10.53 LT, and has relatively weak dissipation rates between 0.3 and 0.9 MPa and on the intrusions where $Ri > 1$.



variability. The cycle is not symmetric, but has maxima lasting longer than the minima. Examination of more detailed plots shows that decreases in ϵ begin nearly simultaneously with depth. Nevertheless, the minimum has a phase lag that increases downward (Fig. 3). At 0.65 MPa, the lag is 3–6 h after local noon.

Between 0.3 and 0.9 MPa the diurnal cycles in ϵ and χ produce large changes in the overturning scales of the turbulence and in J_q (Fig. 4). Two measures of overturning are used: the Ozmidov scale, $L_o \equiv \epsilon^{1/2} N^{-3/2}$, and l_r , the root-mean-square displacement computed by comparing the observed σ_θ profiles with resorted monotonic versions of the same data^{19,20}. The two scales show good agreement. Below 0.3 MPa, the overturning scales at noon are only a few tens of centimetres, but, during the night, they grow to a few metres. Correspondingly, J_q increases from about 30 W m^{-2} at noon to about 240 W m^{-2} at midnight. At night these estimates are unreliable above 0.25 MPa owing to the weak stratification in the mixed layer.

Thermohaline intrusions from 1.0 to 1.5 MPa

Between 1.0 and 1.5 MPa, thermohaline intrusions strongly affect ϵ and χ . Although temperature usually decreases monotonically over scales greater than 1 m, salinity has a sequence of maxima and minima (Fig. 5). These can only be of advective origin, and are probably associated with the salinity front near the Equator²¹. Changes between successive profiles indicate that the intrusions have horizontal scales less than a few kilometres. Very high dissipation rates occur beneath the salinity maximum of some intrusions. For example, comparing Fig. 5 with the average profiles in Fig. 2 shows that between 1.3 and 1.4 MPa ϵ is as high as $\bar{\epsilon}$ at 0.3 MPa, and χ is higher than $\bar{\chi}$. A similar pattern, found on thermohaline intrusions in a warm-core ring, was attributed to a combination of salt fingering and the shear of near-inertial motions²². Because strong lateral processes, and possibly salt fingering, are involved, neither the dissipation method nor the Osborn-Cox model are applicable to the mean profiles in this pressure range.

Discussion

Based on profiles of $\bar{\epsilon}$ and $\bar{\chi}$, five mixing zones are found in the upper 1.5 MPa below the near-surface zone: (1) the diurnal mixed layer, 0.1–0.3 MPa, has high dissipation rates, which are modulated by almost a factor of 100 and are in phase with the surface heat flux; (2) the upper high-shear zone, 0.3–0.9 MPa, has dissipation rates and turbulent fluxes that are high just below the mixed layer but decrease with depth, as Ri increases. At a given depth, the dissipation rates vary by factors of 10 to 100

in a diurnal cycle; (3) the velocity maximum, 0.9 to 1.25 MPa, has low dissipation rates, similar to those in mid-gyre; and (4) the lower high-shear zone, 1.25 to 1.5 MPa, has high dissipation rates when thermohaline intrusions occur.

The patterns and magnitudes of these observations are consistent with previous profiles in the undercurrent and are inconsistent with the towed observations. Discovery of the diurnal cycle in the stratified region below the surface mixed layer and of the role of thermohaline intrusions in the dissipation below the core is new.

The daily ϵ cycle is nearly as strong between 0.3 and 0.65 MPa as in the diurnal mixed layer. This is a surprise in view of the restricted depth range of the diurnal cycle in stratification. N variations are readily apparent (Fig. 3), but they have no diurnal signature similar to that in ϵ and χ . To our knowledge no previous observations have been reported, from any location, of a diurnal cycle below the mixed layer and its entrainment zone.

We have considered several possible causes of the deep diurnal mixing cycle: the absorption of solar radiation, a diurnal cycle in the large-scale shear, and daily modulation of high-frequency internal waves.

Suppression of the turbulence by a buoyancy flux resulting from the absorption of solar radiation seemed a likely mechanism when we noticed the diurnal cycle at sea and has since been suggested to us by several others (M. Lewis and J. D. Woods, personal communications). We do not, however, understand how this can happen without also producing a daily cycle in N , which is not apparent in Fig. 3 or in other more detailed plots.

No diurnal shear cycle has been reported from the Equator; but, in response to discovery of the diurnal mixing cycle, current meter records from moorings at 0°N , 140°W in May and November 1983 have been examined (D. Halpern, personal communication). Between 0.1 and 0.25 MPa, shear has a strong daily fluctuation, $\sim 0.01 \text{ s}^{-1}$, but this decreases rapidly with depth and is negligible below the diurnal mixed layer, especially compared with the semi-diurnal tidal signal.

Internal wave energy on the equator is higher than found in mid-gyre²³, and laboratory studies show that mixed layer turbulence can generate internal waves in the thermocline²⁴. Therefore, internal waves, generated when turbulence is strong in the mixed layer, may propagate downward and break in the stratified high-shear zone, producing much of the observed mixing. When the surface layer restratifies during the day the source of internal waves will be suppressed, leading to a minimum in mixing below the mixed layer which may have a phase lag as the effect propagates downward.

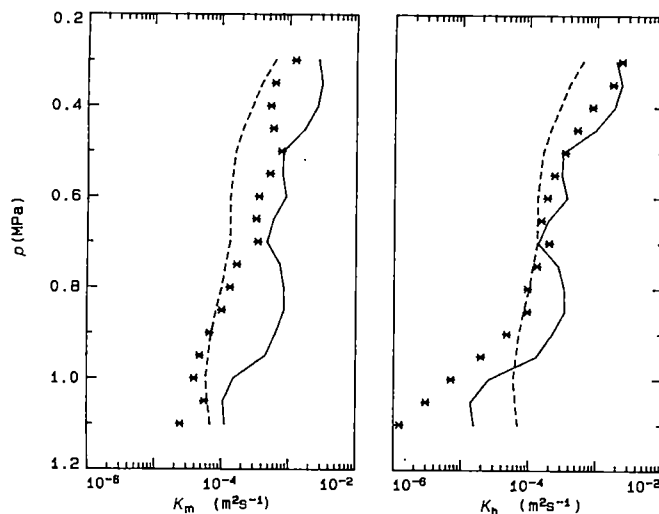


Fig. 6 Comparison of K_m and K_h obtained using the dissipation method (*) with parameterizations used in two numerical models of the undercurrent. Dashed line, from ref. 15; solid line, from ref. 16. The model K_m and K_h are functions of the observed $\bar{N}(p)$ and/or $Ri(p)$. The parameterizations are closer where Ri is low, above 0.7 MPa, than where Ri is high.

The data available to us are inadequate to determine whether one of these mechanisms is the cause, because small changes in either N or shear could be responsible. Mixing in this zone resembles a sharply tuned harmonic oscillator, which can have large output changes for small forcing perturbations. Plotting the $\bar{\epsilon}$ profile as a function of Ri shows a large increase when Ri drops below 0.25. Consequently, a 20% increase in shear or a similar decrease in N can change the average Richardson number from 0.35 to 0.25. Figure 3 shows much larger changes occurring in N , but with no apparent daily frequency. The eight XCP profiles show that the shear also changes by more than 20%. Because a wide band of frequencies seem to contribute to changes in both N and shear, longer time series of both are needed to define a diurnal signature. Data taken by other Tropic Heat investigators may be able to resolve this, but we know of no measurements that can examine the role of high-frequency internal waves and recommend that such measurements be included in future experiments.

In addition to taking the time series on the Equator we made measurements at 0.5° intervals off the Equator and found that the diurnal cycle extended to at least 1.0 MPa just outside of the undercurrent, compared with 0.3 MPa, in the centre of the undercurrent. It is possible that internal waves radiated from these deeper layers are also involved and that the problem is three-dimensional rather than just one-dimensional.

Two recent numerical models of the undercurrent are based on previous microstructure observations. One parameterizes the eddy coefficients in terms of N , largely for mathematical convenience¹⁵

$$K_m = K_h = AN^{-2} \quad (5)$$

with A chosen to produce $K_m = 5.5 \times 10^{-5} \text{ m}^2 \text{ s}^{-1}$ at N_{max} . Another¹⁶ bases the parameterization on Ri

$$K_m = 1 \times 10^{-4} + \frac{5.5 \times 10^{-3}}{(1 + 5 Ri)^2} \quad (6)$$

and

$$K_h = 1 \times 10^{-5} + \frac{K_m}{1 + 5 Ri} \quad (7)$$

Comparison with our estimates, using equations (1) and (3), shows that equation (6) is consistently high, except between 0.5 and 0.7 MPa (Fig. 6). Equation (7) agrees remarkably well with observations above 0.7 MPa, but is an overestimate at greater depths. Equation (5) agrees relatively well with observations for K_m , but is low by factors of 2–5 between 0.4 and 0.7 MPa. For K_h , equation (5) has much less vertical change than the observations, and crosses from an under to an over estimate near 0.7 MPa. At 1.1 MPa, it is almost a factor of 100 too high.

Both parameterizations are in rough agreement with K_m and K_h computed from our data and equations (1) and (3), but neither is a good fit. The major discrepancies are overestimation of both eddy coefficients at the velocity maximum (the additive terms are much too large) and underestimation of the increases in K_m and K_h when Ri drops below 0.25. A plot of K_m and K_h as functions of Ri shows that they rise abruptly by about a factor of 10 when Ri approaches 0.25. Because a large section of the mixing region is in this Richardson number range, improving this part of the parameterization is particularly important.

Until the diurnal cycle is understood, these parameterizations, which are based on only the large-scale profiles, must be viewed as tentative. If fluctuating internal wave energy or the absorption of solar energy has a major role, a simple Ri parameterization will be inadequate. Discovery of the diurnal cycle also raises the possibility of an annual cycle, because the intensity of the diurnal cycle may vary with season.

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Hotspot magmas can form by fractionation and contamination of mid-ocean ridge basalts

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Melts ascending from deep reservoirs and trapped beneath thick lithosphere exchange heat with and partially melt the shallow mantle as they crystallize. Basalts erupting through thick lithosphere are more likely to have experienced deep crystal fractionation and contamination than those at mature spreading centres. Geochemical data for ocean island and other hotspot magmas do not require a primitive or lower mantle source.

MID-OCEAN ridge basalts (MORB) are the most depleted (low concentrations of large-ion lithophile (LIL) elements, low values of Rb/Sr, Nd/Sm, $^{87}\text{Sr}/^{86}\text{Sr}$, $^{144}\text{Nd}/^{143}\text{Nd}$, $^{206}\text{Pb}/^{204}\text{Pb}$) and the most voluminous magma type. They erupt through thin lithosphere and have apparently experienced some crystal fractionation before eruption. Isotope ratios show that the MORB reservoir was outgassed and depleted more than 10^9 yr ago. There is growing evidence that there is also an ancient enriched reservoir in the mantle and that the continental crust is not the only complement to the MORB reservoir¹⁻⁴. The most enriched mantle magmas are lamproites, kimberlites, and basalts from islands such as Kerguelen, St Helena and Tristan da Cunha. There is a complete spectrum of basalts lying between these extremes. The presence of ^3He in MORB and the fact that its $^3\text{He}/^4\text{He}$ is higher than atmospheric^{5,6} suggests that outgassing was not 100% efficient, or that MORB is contaminated. Pb isotopes suggest that MORB has experienced some contamination before eruption⁷. Therefore, MORB itself may be a hybrid magma.

The geochemistry of various magma types requires at least two distinct reservoirs in the mantle. The relatively uniform depleted mantle (DM) suffered an ancient depletion event, presumably by removal of a residual fluid. The other reservoir, which we call enriched mantle (EM), is less homogeneous and is presumed to provide the enriched signature of hotspot magmas. Correlated LIL and isotope variations in mantle magmas are often explained by mixing of material from the EM and DM reservoirs.

Basalts with high Rb/Sr, La/Yb and Nd/Sm ratios generally have high $^{87}\text{Sr}/^{86}\text{Sr}$ and low $^{143}\text{Nd}/^{144}\text{Nd}$ ratios. These can be explained by binary mixing of depleted and enriched magmas or by mixtures of a depleted magma and a component representing varying degrees of melting of an enriched reservoir⁷. The variable LIL ratios of such an enriched component generate a range of mixing hyperboles or 'scatter' about a binary mixing curve, even if there are only two isotopically distinct end members. Thus a model with two isotopically distinct reservoirs can generate an infinite variety of mixing lines⁷. In some regions, however, the inverse relationship between LIL and isotope ratios cannot be explained by binary mixing⁸⁻¹³. These regions are all in midplate or thick lithosphere environments and sublithospheric crystal fractionation involving garnet and clinopyroxene might be expected before eruption.

The wide range of isotope ratios in island and continental basalts has led to the view that the mantle is heterogeneous on all scales. The failure of simple binary mixing to explain the spread of isotope and trace element ratios, and the presence of LIL-enriched basalts with time-integrated depleted isotope ratios, has also given rise to the concept that recent mantle metasomatism has affected the source region and may even be a prerequisite for magmatism¹⁴.

Some of the observations which have been interpreted in terms of metasomatism, or an extremely heterogeneous mantle are: (1) scatter of $^{143}\text{Nd}/^{144}\text{Nd}$ - $^{87}\text{Sr}/^{86}\text{Sr}$ values about the mantle array; (2) lack of correlation between $^{87}\text{Sr}/^{86}\text{Sr}$ and $^{206}\text{Pb}/^{204}\text{Pb}$

or $^4\text{He}/^3\text{He}$; and (3) inverse correlations between La/Ce or Rb/Sr and $^{87}\text{Sr}/^{86}\text{Sr}$. However, if one or both end members of a hybrid is a fractionated melt, or represents varying degrees of partial melting, then these observations do not necessarily require multiple sources. If melts from one reservoir must traverse the other reservoir on their way to the surface there is no need for a 'plum pudding' mantle or recent metasomatism of the source.

The cases where a depleted magma causes variable degrees of melting of an enriched reservoir or an undepleted magma partially melts a depleted layer have already been treated^{7,8}. We now treat a fractionating depleted magma interacting with an enriched reservoir. As a point of reference, we consider the depleted magma from DM to be the parent of MORB. If this magma is brought to a near-surface environment it may fractionate olivine, plagioclase and orthopyroxene. If arrested by thick lithosphere it will fractionate garnet (gt) and clinopyroxene (cpx). We assume that the fractionating crystals and melt are in equilibrium. We emphasize varying degrees of crystal fractionation but our results are applicable to the case where the depleted component represents varying degrees of partial melting⁸ of a gt- and cpx-rich region, such as garnet pyroxenite lower oceanic lithosphere, or an eclogite slab.

There are two situations that have particular relevance to mantle-derived magmas. Consider a 'normal' depleted mantle magma. If it can rise unimpeded from its source to the surface, such as at a rapidly spreading ridge, we have a relatively unfractionated, uncontaminated melt, that is, MORB. Suppose now that the magma rises in a midplate environment, and its ascent is impeded by thick lithosphere. The magma will cool and crystallize, simultaneously partially melting or reacting with the surrounding shallow mantle. Thus, crystal fractionation and mixing occur together, and the composition of the hybrid melt changes with time and with the extent of fractionation. Fractionation of gt and cpx from a tholeiitic or picritic magma at sublithospheric depths (>50 km) can generate alkalic magmas with enriched and fractionated LIL patterns. We investigate the effects of combined eclogite fractionation (equal parts of gt and

Table 1 Parameters of endmembers

	Depleted mantle 1	Enriched mantle 2	Enrichment ratio
$^{87}\text{Sr}/^{86}\text{Sr}$	0.701, 0.702	0.722	Sr_2/Sr_1 13.8
ϵ_{Nd}	24.6	-16.4	Nd_2/Nd_1 73.8
$^{206}\text{Pb}/^{204}\text{Pb}$	16.5, 17.0	26.5	Pb_2/Pb_1 62.5
$^3\text{He}/^4\text{He}$	$6.5 R_a$	$150 R_a$	He_2/He_1 2
La/Ce	0.265	0.50	Ce_2/Ce_1 1 65
Sm/Nd	0.50, 0.375	0.09, 0.139	
Partition coefficients			
Rb	0.02	Sr 0.04	La 0.012
Ce	0.03	Nd 0.09	Sm 0.18
Pb	0, 0.002	He 0.50	

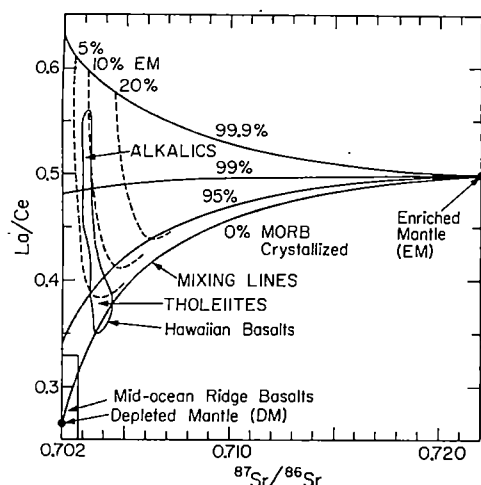


Fig. 1 La/Ce versus $^{87}\text{Sr}/^{86}\text{Sr}$ for the fractionation-contamination model, compared with Hawaiian basalts. Unfractionated MORB has La/Ce = 0.265. La/Ce of the depleted endmember increases as crystal fractionation proceeds. The enriched endmember has La/Ce = 0.5, in the range of kimberlitic magmas. The Hawaiian tholeiites can be modelled as mixes ranging from pure MORB plus 2–7% enriched component to melts representing residuals after 95% cpx plus gt crystal fractionation and 5–8% enriched component. Alkali basalts involve more crystallization and more contamination. In all the figures, solid curves are mixing lines between EM and melts representing fractionating depleted magmas. Dashed curves are trajectories of constant mixing proportions. Data from ref. 8.

cpx) and 'contamination' on melts from DM. 'Contamination' is modelled by mixing an enriched component with the fractionating depleted magma. This component is viewed as a partial melt generated by the latent heat associated with the crystal fractionation. The assumed geochemical properties of the endmembers, the enrichment factors of the elements in question and the partition coefficients, D , assumed in the modelling are given in Table 1. D for Rb, Sr, Sm, Nd, La and Ce are the mean for cpx and gt¹⁵. Later we shall discuss Pb and He and, in these cases, D is an adjustable parameter. The figures show various ratios for mixes of a fractionating depleted melt and an enriched component (EM). I assume equilibrium crystal fractionation as appropriate for a turbulent or permeable magma body, and constant D . I will not attempt to fit the data displayed in the figures. My purpose is simply to show the range of parameters required to span the data by the proposed mechanism. I do not rule out other contributions to the geochemical complexity of magmas. In particular, I shall discuss the heterogeneity of EM.

La/C versus $^{87}\text{Sr}/^{86}\text{Sr}$

La/Ce is high in melts relative to crystalline residues containing gt and cpx. It is low in depleted reservoirs and high in enriched reservoirs. Low and high values of $^{87}\text{Sr}/^{86}\text{Sr}$ are characteristics of time-integrated depleted and enriched magmas, respectively. As high $^{87}\text{Sr}/^{86}\text{Sr}$ implies a time-integrated enrichment of Rb/Sr, there is generally a positive correlation of Rb/Sr and La/Ce with $^{87}\text{Sr}/^{86}\text{Sr}$. Some magmas⁹, however, exhibit high La/Ce and low $^{87}\text{Sr}/^{86}\text{Sr}$. This cannot be explained by binary mixing of two homogeneous magmas.

On a theoretical La/Ce versus $^{87}\text{Sr}/^{86}\text{Sr}$ plot (Fig. 1) the mixing lines between the crystallizing MORB and EM components reverse slope when MORB has experienced slightly more than 99% crystal fractionation. The relationships for equilibrium partial melting and equilibrium, or batch, crystallization are, of course, the same. Therefore, large degrees of crystallization of a MORB-like melt or small amounts of partial melting of a MORB layer are implied by an inverse relationship between La/Ce (or Rb/Sr, La/Yb, Nd/Sm, and so on) and $^{87}\text{Sr}/^{86}\text{Sr}$ (or $^{144}\text{Nd}/^{143}\text{Nd}$) such as observed at many mid-plate

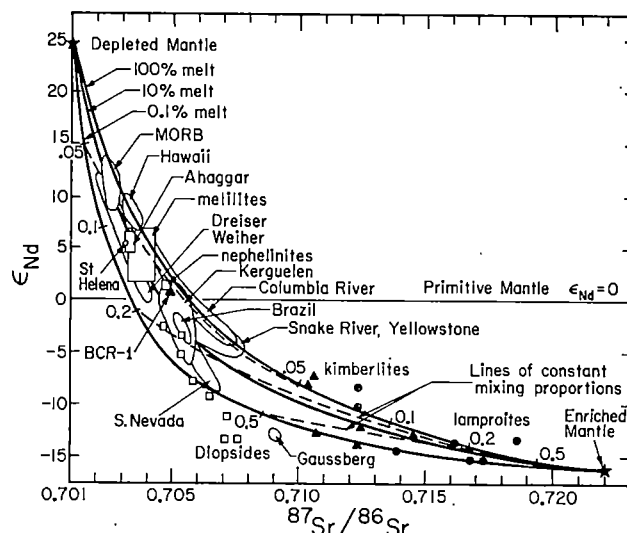


Fig. 2 ϵ_{Nd} versus $^{87}\text{Sr}/^{86}\text{Sr}$ for mixtures involving a depleted magma or residual fluids from such a magma after crystal fractionation, and an enriched component (EM). Data from various sources including refs 4, 38–44.

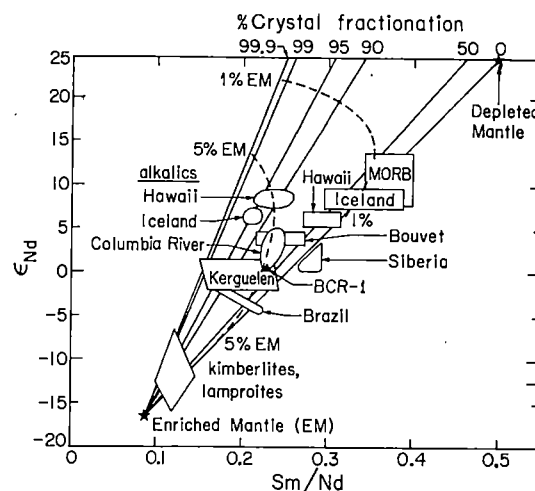


Fig. 3 $^{143}\text{Nd}/^{144}\text{Nd}$ (relative to primitive mantle) versus Sm/Nd for mixtures of enriched mantle and residual melts resulting from high-pressure crystal fractionation of MORB-like depleted magma. Data from refs 8, 15, 38–40. See ref. 45 for supporting evidence for Iceland.

environments. The apparently contradictory behaviour of magmas with evidence for current enrichment and long-term depletion is often used as evidence for 'recent mantle metasomatism'. Figure 1 illustrates an alternative explanation. Note that, with the parameters chosen, the Hawaiian alkalis have up to 10% contamination by the enriched component.

ϵ_{Nd} versus $^{87}\text{Sr}/^{86}\text{Sr}$

Isotope ratios for ocean island and continental basalts are compared with mixing curves in Fig. 2. These basalts can be interpreted as mixes between a fractionating depleted magma and an enriched component. The value for primitive mantle ($\epsilon_{\text{Nd}} = 0$) is also shown. The primitive mantle value of $^{87}\text{Sr}/^{86}\text{Sr}$ is unknown and cannot be inferred from basalts that are themselves mixtures. For example, the basalts cover a field extending from 0 to 99.9% crystal fractionation and the corresponding $^{87}\text{Sr}/^{86}\text{Sr}$ ratios at $\epsilon_{\text{Nd}} = 0$ range from 0.7043 to 0.7066. The extreme situations, constant degree of fractionation combined with variable contamination, and constant mixing proportions combined with variable fractionation, can each explain the trend of the data. Together, they can explain most of the dispersion of the data.

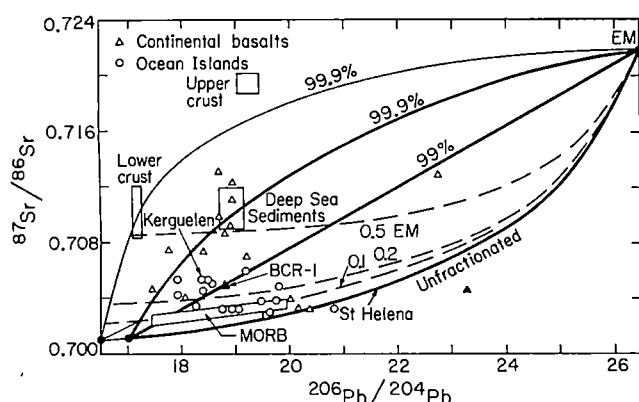


Fig. 4 $^{87}\text{Sr}/^{86}\text{Sr}$ versus $^{206}\text{Pb}/^{204}\text{Pb}$ trajectories for mixtures of a fractionating depleted MORB-like magma and an enriched component. Mixing hyperboles are solid lines. Hawaiian basalts fall in the region of 50–99% crystal fractionation (garnet plus clinopyroxene) and 10–30% contamination (EM). Data from various sources including refs 23, 46–49.

ϵ_{Nd} versus Sm/Nd

The mixture-fractionation curves for this system are shown in Fig. 3. High Sm/Nd basalts from Iceland, Hawaii, Siberia, Kerguelen and Brazil all fall near the curve for unfractionated MORB with 1–5% contamination. Alkalics from large oceanic islands with thick crust (Hawaii, Iceland and Kerguelen) are consistent with large amounts of crystal fractionation and moderate (5–10%) amounts of contamination.

The interpretation is that the more voluminous tholeiites comprise slightly fractionated and contaminated MORB, while the alkalics have experienced sublithospheric crystal fractionation and contamination before eruption. MORB itself has ~1% contamination, similar to that required to explain Pb isotopes⁷. The Hawaiian tholeiites can be modelled as MORB that has experienced variable degrees of deep crystal fractionation (0–95%), mixed with 1–5% of an enriched component. The alkali basalts represent greater extents of crystal fractionation and contamination. The Columbia River basalts can be interpreted as MORB which has experienced 80% crystal fractionation and 5% contamination.

Lead and helium isotopes

Rb, Sr and the light rare earth elements (REE) are classic incompatible elements and the effects of partial melting, fractionation and mixing can be explored with some confidence. Relations between these elements and their isotopes should be fairly coherent. Some enriched magmas also have high $^3\text{He}/^4\text{He}$, $\delta^{18}\text{O}$ and $^{206}\text{Pb}/^{204}\text{Pb}$. These isotopes provide important constraints on mantle evolution, but they may be decoupled from the LIL variations and are usually assumed to be so. $^3\text{He}/^4\text{He}$ depends on the outgassing and magmatic history of the depleted reservoir and the U and Th content and age of the enriched component. $^{206}\text{Pb}/^{204}\text{Pb}$ and $^3\text{He}/^4\text{He}$ are sensitive to the age of various events affecting a reservoir because of the short half life of U. The U/Pb ratio may be controlled by sulphides and metals as well as silicates. Nevertheless, it is instructive to investigate the effects of contamination and fractionation on these systems to seek out what other effects or components are involved.

There is no simple relationship between the Sr and Pb isotopes in mantle magmas. A minimum of three components is required to satisfy both isotope systems when only simple mixing is considered. St Helena, for example, has high $^{206}\text{Pb}/^{204}\text{Pb}$ –low $^{87}\text{Sr}/^{86}\text{Sr}$, Kerguelen has high $^{87}\text{Sr}/^{86}\text{Sr}$ –low $^{206}\text{Pb}/^{204}\text{Pb}$, and MORB has low $^{87}\text{Sr}/^{86}\text{Sr}$ –low $^{206}\text{Pb}/^{204}\text{Pb}$. The Pb isotopes in MORB are higher than expected for a primitive or depleted

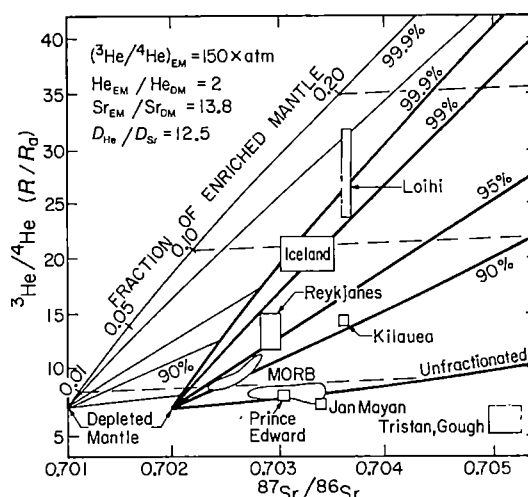


Fig. 5 Mixing curves for $^3\text{He}/^4\text{He}$ versus $^{87}\text{Sr}/^{86}\text{Sr}$. Two choices for the depleted endmember are shown. Hawaiian basalts imply 90 to >99% crystal fractionation and 5–18% contamination. To make Iceland consistent with Fig. 3, one could raise Sm/Nd (DM) or R_a (EM). Data from refs 50–53.

reservoir. Figure 4 shows a mixing-fractionation curve for $^{87}\text{Sr}/^{86}\text{Sr}$ and $^{206}\text{Pb}/^{204}\text{Pb}$. To span the data, with only two distinct endmembers, it has to be assumed that the partition coefficient for Pb is much less than that for Sr. This would be the case if Pb is entirely contained in low-melting-point phases or is much more incompatible in gt and cpx than is Sr. Figure 4 shows that the data can be explained with only two isotopically distinct mantle reservoirs. However, even if EM is global it can have variable $^{206}\text{Pb}/^{204}\text{Pb}$ and $^{207}\text{Pb}/^{204}\text{Pb}$ as these are sensitive to the U/Pb ratio and the age of enrichment events. U/Pb and $^3\text{He}/^4\text{He}$ of EM will change locally by the subduction of sediments and oceanic crust and by the insertion of basalts which do not make their way to the surface. Isotope ratios of Pb and He, therefore, are variable and change rapidly with time. The various contributions to the enrichment process (subduction, trapped magmas) make it likely that EM, the shallow mantle in our case, is laterally inhomogeneous.

Mid-ocean ridge tholeiites¹⁶ average about 8.5 times the atmospheric ratio (R_a) for $^3\text{He}/^4\text{He}$. Samples from Hawaii^{17,18} have R_a up to 37 and natural diamonds^{19,20} range up to 280. $^3\text{He}/^4\text{He}$ near Iceland and Hawaii can be modelled as two-component mixtures with MORB and Loihi being the endmembers. Other islands have lower ratios than MORB, suggesting a third component, a variably outgassed EM or reduction in $^3\text{He}/^4\text{He}$ by U–Th decay. The high $^3\text{He}/^4\text{He}$ of some ocean island basalts is often assumed to require decoupling from other processes and a lower mantle origin.

Results are shown in Fig. 5. The highest $^3\text{He}/^4\text{He}$ samples from Loihi imply ~18% of the enriched endmember ($R_a = 150$) and >99% crystal fractionation. Prince Edward and Jan Mayan can be modelled as mixtures involving nearly unfractionated MORB. Basalts from Tristan and Gough may require a third component, such as subducted sediments, or they may have evolved in a high U–Th environment. To cover the total range with the chosen endmembers, it is necessary to assume that the He abundance in EM is about twice the abundance in the depleted magma and that D for He is much greater than that for Sr. The partition coefficient for He is unknown, but it may not behave as a classic incompatible element. Clinopyroxene-melt 'partition' (or 'trapping') coefficients for Ar and Kr are 0.2–0.5, and may increase with decreasing atomic weight²⁰. These surprisingly high values are of the order required to explain the He–Sr isotope results. The distribution of $^3\text{He}/^4\text{He}$ in the enriched reservoir is likely to be uneven because it depends on the amount of trapped ^3He , the U and Th content, and the age of enrichment. If part of the LIL enrichment is due to reinjection of oceanic crust and sediments, this part of EM will have a low

$^3\text{He}/^4\text{He}$ ratio. Ancient enriched reservoirs will have higher $^{206}\text{Pb}/^{204}\text{Pb}$ and lower $^3\text{He}/^4\text{He}$ ratios than equivalent modern reservoirs. Nevertheless, combined crystal fractionation and contamination, using only two endmembers, can explain much of the range and several of the trends observed in oceanic basalts.

Discussion

Chen and Frey⁸ propose a model for Hawaiian volcanism that involves melting of an enriched mantle plume from the lower ('primitive') mantle. This upwelling plume traverses a depleted (MORB) region, which partially melts (0.05–2.0%); these melts mix in varying proportions to form the spectrum of basalts found in Hawaiian volcanoes. With their choice of endmembers the MORB component must be small, <2%.

Our results suggest an alternative explanation. Melts from DM rise to the base of the lithosphere, fractionate and mix with melts from EM. At mid-ocean ridges the lithosphere is thin, crystal fractionation occurs at shallow depths, and eruption occurs with little contamination. Mid-plate volcanism involves crystal fractionation at greater depth, >60 km. Because of slower cooling at depth, there is more chance for mixing with melts from the asthenosphere and lithosphere before eruption. Thus, diapirs from the depleted reservoir may be the precursors for both mid-ocean and mid-plate volcanism, but the trace-element and isotope spectra of basalts depend on the thickness of the lithosphere or crust. Material from DM, which initiates melting in the shallow enriched reservoir, dominates the mix. The choice of a single EM component is simplistic but it illustrates the mechanism. On their ascent, MORB diapirs can interact with asthenosphere, oceanic or continental lithosphere, sediments, sea water and subducted material.

In the Chen–Frey mode, the amount of the MORB component decreases as the inferred degree of partial melting of the MORB source increases, the reverse of what might be expected. The relative portions of the MORB and enriched components are also the reverse of what the $^3\text{He}/^4\text{He}$ data indicate. Kaneoka²¹ suggests that the MORB component of Hawaiian basalts represents more than 50–70%. In the present model, the amount of contamination increases as crystal fractionation proceeds.

Kaneoka²¹ favours a model in which LIL- and ^3He -rich material from a deep primitive mantle reservoir rises through and mixes with a shallow, depleted region. He argues that if the enriched layer is shallow, plumes should originate at shallow depth, and the only overlying depleted layer is the oceanic lithosphere. However, the distinctive geochemical signature of hotspot basalts may be acquired at shallow depths, and most of the erupted material may be depleted basalts rising from greater depth. Continental contamination is an oft-invoked mechanism of this type, although it does not work for the rare gases. An LIL- and ^3He -rich shallow mantle does not imply that plumes originate at shallow depth. In discussions of hotspots, it should be recognized that the source of the heat, the source of most of the magma and the source of the isotope signatures may be at different depths. Hot regions of the lower mantle, for example, can localize melting in the transition region (400–650 km depth) and depleted melts from this region may become contaminated as they rise. Melts trapped in, or rising through, the shallow mantle may be responsible for the distinctive physical properties of the low-velocity zone and asthenosphere. Most workers assume that noble gases are strongly partitioned into melts which then efficiently lose these gases to the atmosphere. Outgassing, however, probably occurs only at relatively shallow depths, where the major gases, such as CO_2 and H_2O , can exolve²². If melts are trapped at depth, the noble gases will be as well. The presence of ^3He and ^{36}Ar in magmas does not require the existence of a previously unmelted or primitive reservoir. The $^3\text{He}/^4\text{He}$ characteristics of hotspot magmas, therefore, do not require an explanation separate from that for the other isotopes, except that $^3\text{He}/^4\text{He}$ can decrease rapidly with time in a high-U reservoir.

The magnitude of tholeiitic volcanism, the dominance of the MORB component in mantle magmas and the close temporal

and spatial relationship of depleted and more enriched and evolved alkalic magmas support a controlling role for melts from DM in mantle magma genesis. I propose that oceanic ridges, islands and seamounts share a common source and that mantle magmas differ primarily because of different degrees of sublithospheric crystal fractionation and contamination, and the nature of the (minor) enriched component. Shallow fractionation, crustal contamination, alteration and recycling, will be superimposed on the effects considered here. There is also the possibility that EM is inhomogeneously enriched or veined. Pb isotopes, for example, require more than two endmembers²³. These may differ in age or intrinsic chemistry, or both.

The location of EM, the source of the contamination discussed here, cannot be settled by petrological and geochemical arguments alone. It has been a common, but unfortunate, practice in the recent geochemical literature to avoid the issue by using the terms lower mantle, primitive mantle, undegassed mantle, plume reservoir and ocean island reservoir, interchangeably. The similarity in trace-element and isotope geochemistry between large islands, seamounts, continental rifts and island arcs suggests that EM is widespread and shallow. The most enriched magmas, kimberlites, appear to originate at a depth of ~200 km. Mid-ocean ridges can be traced by seismic means to depths >200 km, in some cases, >400 km (refs 24, 25). The ascent phase of a deep diapir is characterized by melting. Crystallization is caused by entry of the diapir into the cold surface boundary layer. The heat of crystallization causes melting, or wall-rock reactions, and, hence, crystal fractionation and contamination are concurrent processes. The adoption of a single-stage process involving two isotopically distinct endmembers, only one of which is allowed to fractionate, is clearly an oversimplification. The enriched component may represent variable degrees of partial melting of an enriched region which is probably inhomogeneous. The shallow mantle is periodically replenished with subducted material and trapped magmas. Some of these processes serve to diminish the extreme amount of crystal fractionation implied by the present calculations, as can non-Henry's Law behaviour^{26,27}. The possibility of large amounts of eclogite or clinopyroxene fractionation has been discussed by O'Hara²⁸ and others^{29–36}.

There are several mechanisms by which EM might be formed: (1) Melts removed from DM are distributed between the crust and the shallow mantle. (2) Reinjection of sediments and crust (recycling). In both cases, EM is expected to be shallow. The melt and crustal extraction processes are not 100% efficient; thus, LIL-rich melts are trapped in the shallow mantle. Sediments and altered oceanic crust, the top part of the subducted system, are exposed to high temperatures and stresses so they may dehydrate, melt and be scraped off at shallow depths. This component is low in ^8He .

A thin, shallow layer is unlikely to be globally homogenized by plate tectonic processes. The large-scale isotope anomaly in the Southern Hemisphere mantle³⁷ may have been the locus of ancient subduction, just as the circum-Pacific belt is today or the circum-Pangea belt was in the Cenozoic. In this case the Dupal³⁷ anomaly would be due to an ancient subducted component in the shallow mantle (high ^{87}Sr , ^{206}Pb , ^4He). Such an anomaly would be smeared out in time, in the direction of mantle flow, but would diffuse only slowly across the upwellings and downwellings which separate upper mantle convection cells, particularly if it is embedded in buoyant material such as harzburgite.

Note that my model contradicts conventional views of hotspot petrogenesis. The enriched reservoir is shallow rather than deep and it may be relatively infertile, that is, low in CaO and Al_2O_3 . Being shallow it can collect volatiles and LIL by subduction and by trapping of fluids rising from below. Although global it is unlikely to be homogeneous. It protects the deeper, depleted but fertile (low LIL, high CaO and Al_2O_3) reservoir from contamination. It is usually assumed that the source of heat, the source of the basalts and the source of the hotspot geochemical signatures are all the same. The relative stationariness of hot-

spots, the associated swells and high geoid may be due to increased melting in the upper mantle caused by locally high lower mantle temperatures rather than transfer of material from the lower mantle to the surface. It is the thickness of the lithosphere that controls the geochemical signature of intraplate volcanics, not the ease of communication to the lower mantle.

The lower mantle, other than being a source of heat, may not be involved at all in petrogenesis.

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Expression of a foreign gene in myeloid and lymphoid cells derived from multipotent haematopoietic precursors

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Bone marrow cells infected with retroviral vectors carrying the bacterial neomycin resistance (neo) gene as a marker were used for long-term reconstitution of the haematopoietic system of irradiated mice. The neo gene is expressed in the myeloid and lymphoid lineages of these animals and an analysis of the sites of viral integration indicates that these lineages are derived from the same primitive multipotent cells.

THE transfer of foreign genes with retroviral vectors to precursors of the haematopoietic system provides a unique opportunity to analyse the expression and function of a variety of different genes in a well-characterized developmental system that can be manipulated both *in vitro* and *in vivo*. In addition, the proviral integration site can be used as a clonal marker for identifying haematopoietic precursors and for following their progeny through differentiation. This is of great interest as our knowledge of the structure of the haematopoietic system, particularly the relationship of the lymphoid lineages to one another or to the other (myeloid) blood cell lineages, is still incomplete. Colony assays have defined a variety of different precursors, including those which are pluripotent, oligopotent and unipotent for the myeloid lineages¹⁻⁵. However, demonstration of the more primitive precursors capable of generating both lymphoid and myeloid progeny, as well as of precursors restricted to the lymphoid lineages, has been more difficult.

Unique chromosome marker experiments⁶⁻⁸ and embryo reconstitution studies⁹ in the mouse as well as isoenzyme analysis in patients suffering from chronic myelogenous leukaemia

(CML)¹⁰ all support the concept of a common precursor for the lymphoid and myeloid lineages. At present, only one report has provided evidence for the existence of long-lived precursors restricted to the T-cell lineage⁸. Precursors committed to the B-cell lineage or lymphoid precursors common to both the B- and T-cell lineages have not yet been described.

Retroviral vectors will be useful for both gene expression and lineage analysis studies, provided that they can infect all classes of precursors and that the transferred genes can be expressed in the progeny of the infected cells. In the present report, retroviral vectors were used to transfer the neomycin resistance (*neo*) gene to a variety of haematopoietic precursors, including those capable of participating in long-term reconstitution of irradiated mice. Expression of the introduced *neo* gene was demonstrated in the different blood cell lineages of all recipients analysed. Unique viral integration sites in the DNA of different tissues, purified cell populations and cloned T- and B-cell hybridomas showed that multipotent precursors capable of generating both lymphoid and myeloid progeny were infected with the recombinant vectors.

Infection of bone marrow cells

In order to achieve a high efficiency of retrovirus infection, the protocol shown in Fig. 1 was developed. The structure of the retroviral vectors (N2 and N3) used for infection is shown in Fig. 2a. N2 was generated helper-free, whereas N3 contains helper virus (Moloney murine leukaemia virus, M-MuLV). The essential features of the protocol include a 24-h co-culture of the bone marrow cells with the virus-producing cells, followed by a 48-h preselection step that enriches for precursors expressing the *neo* gene. In three separate experiments 50–95% of all colony-forming cells (CFC) survived the 24-h co-culture with the virus-producing cells and of these, 10–20% were able to grow and form colonies in the presence of G418. The frequency of G418-resistant (G418^r) CFC was similar following co-culture with cells producing either N2 or N3 virus. Following the preselection step in liquid culture, 15–50% of the input number

of CFC were recovered. However, a high percentage (60–95%) of these were G418^r.

Reconstitution and analysis

Virus-infected cells from unselected and preselected populations were used to reconstitute irradiated recipients. Myeloid and lymphoid tissues from these mice were analysed at different

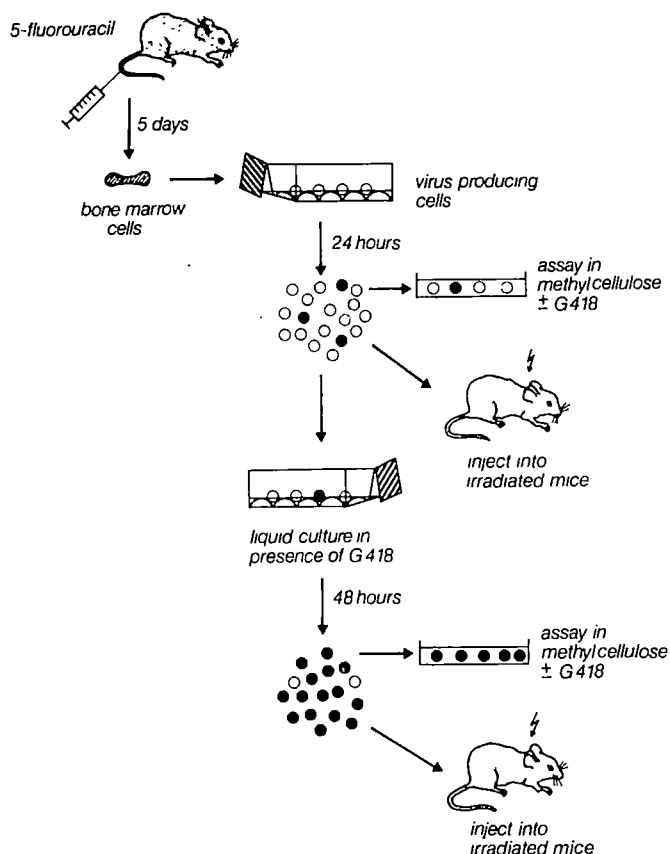


Fig. 1 Protocol for viral infection of bone marrow cells. Bone marrow cells from mice treated 5 days earlier with 5-fluorouracil (150 mg per kg body weight) were seeded directly onto a monolayer of irradiated (1,000 R X-ray) virus-producing cells. CBA-HT6T6Bii mice (>10 weeks old), obtained from the Basel Institute for Immunology breeding colony, were used as a source of donor bone marrow in all experiments. The virus-producing cells were maintained in IMDM with 5% FCS and subcultured the day before infection, to achieve 50–80% confluency on the day of the experiment. For co-culture, half of the supernatant was removed and replaced with fresh medium containing the bone marrow cells, polybrene (10 μ g ml⁻¹), conditioned medium from WEHI-3B (D⁻) cells (20%), transferrin (30 μ g ml⁻¹) and BSA (1 mg ml⁻¹). For reconstituted mice 1, 2, 3 and 4, horse serum (15%) and hydrocortisone (10⁻⁷ M) were also included, whereas for mouse 5 these two reagents were replaced by medium conditioned by adherent bone marrow cells. Either 25-cm² flasks containing 2–3 $\times 10^6$ bone marrow cells in a final volume of 4 ml of medium or 75-cm² flasks containing 5–10 $\times 10^6$ cells in 10 ml of medium, were used. After 24 h of co-culture, the bone marrow cells were collected. A sample from this infected population was assayed in methylcellulose cultures to determine the frequency of G418^r CFC (see Table 1). A second sample was injected into irradiated (900R) recipients (CBA/N; >12 weeks old). The remaining cells were selected in G418 (2 mg ml⁻¹) for 48 h before assaying for (1) G418^r CFC and (2) the ability to reconstitute irradiated recipients. This preselection step was carried out on the virus-producing cells for mice 1 and 2, and in the absence of any adherent layer for mouse 5. WEHI-3B (D⁻) conditioned medium was included in the preselection cultures.

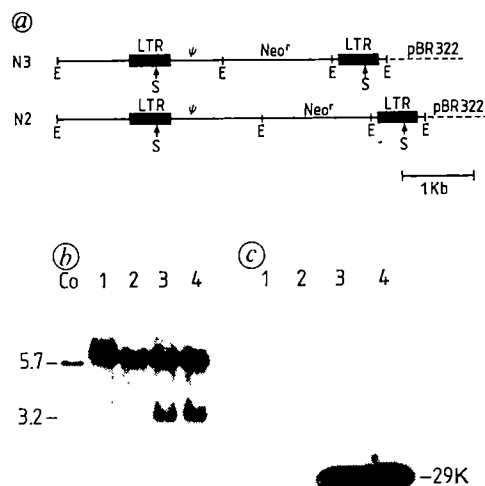


Fig. 2 a, Structure of the *neo*-containing retroviruses. The two constructs (N3 and N2) are composed of three parts: (i) the 5'-long terminal repeat (LTR) fragment containing the viral packaging signal (ψ) was derived from M-MuLV small circular DNA by excision with *Bgl*I and *Xho*I, limited exonuclease digestion with *Bal*31 and addition of *Eco*RI linkers (more viral sequences are present downstream from the 5'-LTR' in N2 than in N3); (ii) the bacterial *neo* gene (*Neo*^r) derived from Tn5 by excision with *Bgl*II and *Bam*HI and addition of *Eco*RI linkers; and (iii) a 3'-LTR fragment which was also derived from M-MuLV small circular DNA by excision with *Bam*HI and *Pst*I, followed by *Bal*31 digestion, addition of *Eco*RI linkers and digestion with *Cla*I. The DNA constructs were generated by standard recombinant DNA techniques, and introduced into a thymidine kinase-negative (TK⁻) derivative of NIH 3T3 cells (N3 virus) or into ψ 2 cells²⁴ (N2 virus) by the calcium phosphate precipitation method. NIH 3T3 or ψ 2 cells were selected with G418 (1 mg ml⁻¹) for 8–10 days. NIH 3T3 cells carrying the N3 provirus were subsequently superinfected with M-MuLV helper virus and supernatants from these cells were harvested and used to infect fresh NIH 3T3 cells. Following G418 selection, single clones were isolated, expanded, and used as a source of recombinant virus. N2-transfected ψ 2 cells were also selected in G418 (1 mg ml⁻¹) and single resistant clones were expanded to produce helper-free virus. N3 was produced at a titre of 10⁶ G418^r colony forming units CFU per ml together with 1–2 $\times 10^6$ XC units of helper virus. N2 was produced helper-free at 5 $\times 10^5$ G418^r CFU ml⁻¹. Supernatants from NIH 3T3 cells infected with N2 virus did not contain G418^r CFU (determined by infection of fresh NIH 3T3 cells). When cells were infected with helper M-MuLV, the supernatant contained 10⁵ G418^r CFU ml⁻¹. This assay can detect the presence of one infectious M-MuLV particle in the N2 virus preparation. b, Analysis of intact proviral DNA sequences in spleen cells from mice that had been reconstituted for 12 days with unselected (equivalent of 850 mix-CFC, 15% G418^r) or preselected (equivalent to 170 mix-CFC, >80% G418^r) cells. DNA of high *M*_r was isolated from the spleens of four reconstituted animals and digested with *Sac*I that cuts once in each of the LTRs of the *neo*-containing and helper proviruses and once within the helper virus genome. Equal amounts (10 μ g) of digested DNA were fractionated on agarose gels, transferred to nitrocellulose by the method of Southern¹², and hybridized with a ³²P-labelled U3-specific probe²⁵. This probe detects the diagnostic 3.2-kb fragment of the intact *neo* provirus and a characteristic 5.7-kb fragment from the helper virus genome. The efficiency of bone marrow infection can be estimated from the band intensities by comparison with the cell line Co, which carries only a single copy of the *neo* gene (2 μ g of DNA was loaded in this lane). Spleen DNAs of two mice, reconstituted with unselected cells, are shown in lanes 1 and 2. Lanes 3, 4 are DNAs from those mice, reconstituted with preselected bone marrow cells. c, NPT-II activity from spleen extracts of the four mice described in b. Prior to DNA extraction, the frozen cell pellets (~10⁷ cells) were thawed and lysed in 200 μ l of H₂O. The suspension was centrifuged for 5 min at 10,000 r.p.m. and the aqueous supernatant was used for the NPT-II assay¹¹. Equal amounts of soluble protein were separated in a non-denaturing polyacrylamide gel, which was then overlaid with a 1% agarose gel containing kanamycin sulphate (100 μ g ml⁻¹) and [γ -³²P]ATP (1.5 μ Ci ml⁻¹). The labelled kanamycin sulphate was transferred onto P81 paper which was washed three times (5 min) with hot water (80 °C), dried and exposed on X-ray film. The labelled 29,000-*M*_r band is diagnostic for the NPT-II activity.

times after reconstitution for expression of the *neo* gene. Expression was demonstrated by the presence of G418^r CFC and by the presence of the *neo* gene product, as determined by the neomycin phosphotransferase (NPT-II) assay¹¹. The presence of the intact proviral structure as well as the existence of unique viral integration sites in these mice was analysed in DNA from the different organs (bone marrow, thymus, lymph node, spleen), from purified cell populations and from hybridomas, by Southern blotting¹².

Table 1 Bone marrow cells used for reconstitution of irradiated mice

Mouse	Pre-selected with G418	Total cell no. injected	No. of mix-CFC injected	% G418 ^r mix-CFC
1	+	2.1 × 10 ⁶	1,350	86
1*	—	5.0 × 10 ⁶	750	26
2	+	2.2 × 10 ⁶	8,100	88
2*	—	5.0 × 10 ⁶	2,200	36
3	—	1.0 × 10 ⁶	2,950	25
4	—	1.0 × 10 ⁶	2,950	25
5	+	4.2 × 10 ⁶	23,100	65
5*	—	5.0 × 10 ⁶	1,150	6
5**	—	5.0 × 10 ⁶	1,170	1

Mice 1–4 were reconstituted with bone marrow cells infected with N3 virus whereas mouse 5 received N2(helper-free)-infected bone marrow cells. No viraemia was detected in the sera of mice 1–4 by a sensitive reverse transcriptase assay despite the fact that competent helper virus was present. Mixed colonies were grown from bone marrow cells in 1-ml cultures (35-mm Petri dishes) containing 0.9% methylcellulose in Iscove's modified Dulbecco's medium (IMDM), 5% fetal calf serum (FCS), 10 mg de-lipidated and deionized bovine serum albumin (BSA), 300 µg iron-saturated transferrin, 22.4 µg dipalmitoyl phosphatidylcholine, 23.5 µg cholesterol and 17.3 µg oleic acid. All reagents were prepared as described by Iscove²⁰. DEAE-Sephrose-purified WEHI-3B(D⁺) conditioned medium as a source of multilineage haematopoietic growth factor (MultiHGF/BPA/IL-3)¹⁴ and serum from anaemic mice as a source of erythropoietin²¹ were added in sufficient amounts to give optimal growth of mixed colonies. Conditioned medium from plastic-adherent bone marrow cells was added (10%) as a source of additional growth factors²². G418 was included at 2 mg ml⁻¹ as indicated. Colonies were counted after 9–12 days at 37 °C in a humidified environment of 5% CO₂ in air. Mixed colony-forming cells (mix-CFC) are those precursors that generate colonies containing cells from the erythroid plus at least two other lineages. * Secondary recipient of bone marrow from indicated mice. ** Tertiary recipient. This animal was not healthy at the time of killing. The thymus and lymph nodes were very small and the marrow was somewhat hypocellular. This is not a surprising finding, as it is well known that the reconstituting capacity of bone marrow decreases dramatically with each passage through an irradiated recipient²³.

Short-term reconstituted mice

The efficiency of infection and preselection was analysed in mice reconstituted for 12 days with N3-infected bone marrow cells. Spleens from both groups contained large numbers of macroscopic colonies. Methylcellulose cultures showed a higher frequency of G418^r CFC in the spleens of mice reconstituted with preselected cells (70%) compared with those that received the unselected population (5%).

To analyse the efficiency of gene transfer and the structure of the provirus, DNAs were prepared from individual spleens of both groups. Southern analysis showed that spleens of mice reconstituted with preselected cells contained more intact *neo*-specific sequences (3.2 kilobases, kb) than DNA from spleens of mice reconstituted with unselected cells (Fig. 2b). Similar amounts of helper virus DNA were found in both groups, although no replicating helper virus was detectable by a reverse transcriptase assay. It is possible that CBA/N mice inhibit viral replication, as others have observed a similar phenomenon (W. Ostertag, personal communication). When cell lysates were tested for an active *neo* gene product, much higher levels of NPT-II activity were found in spleen extracts of mice reconstituted with preselected cells compared with those reconstituted with the unselected population (Fig. 2c).

These findings establish that precursors capable of short-term reconstitution can be infected with the recombinant *neo*-containing virus and can survive the preselection step. In order to establish that primitive precursors capable of long-term reconstitution had been infected with the *neo*-containing virus, we next analysed mice reconstituted for 6–17 weeks. The self-renewal capacity of these precursors was assessed by transferring bone marrow from the primary reconstituted animals (mice 1, 2 and 5) to secondary recipients (1*, 2* and 5*) and in one case to a tertiary recipient (5**). Table 1 lists the different bone marrow populations used for reconstitution.

Long-term reconstituted mice

Expression of the *neo* gene was analysed first in the myeloid lineages by the ability of bone marrow cells from the reconstituted animals to form colonies in methylcellulose cultures containing G418. Independent of the time after reconstitution, all of the animals assayed contained G418^r CFC (Table 2). Those reconstituted with preselected cells had higher frequencies of G418^r CFC (13–37%) than those which received unselected cells (6% and 1% for mice 3 and 4). These findings indicate that the *neo* gene is being expressed in precursors of the myeloid lineages. It is noteworthy that the frequency of G418^r CFC decreased with time in the primary recipient and after transfer of bone marrow to a secondary host (Table 2).

To determine whether or not the *neo* gene was present and expressed in cells of the B-lymphoid lineage, clonable B cells

Table 2 G418-resistant colony-forming cells in reconstituted mice

Mouse	Time of reconstitution (weeks)	Colonies per 10 ⁶ cells						% Total G418 ^r CFC
		-G418			+G418			
		E	Mix	Total	E	Mix	Total	
1	7	220	150	2,470	60	40	780	32
1*	3	—	—	880	—	—	180	21
2	6	490	420	4,150	110	150	1,530	37
2*	4	53	40	963	—	—	100	10
3	9	450	490	3,570	30	20	200	6
4	17	240	470	2,470	2	4	20	1
5	11	310	360	2,370	34	21	305	13
5*	5	125	230	1,425	2	3	19	1
5**	7	130	80	1,900	0	0	3	0.2

Conditions for growth of colonies as described for Table 1. Colonies were classified as follows: E, colonies in which all or nearly all of the cells are erythroid; Mix, mixed colonies containing cells from the erythroid plus at least two other lineages; Total, all colonies in the culture, including E, mix, plus those comprised of mast cells, neutrophils, macrophages and mixtures of these. There were no detectable differences in the appearance of colonies or in the state of maturation of the cells within the colonies in the presence or absence of G418 (2 mg ml⁻¹). The mean numbers of colonies counted in 2–6 replicate cultures are shown. Most long-term reconstituted mice had normal numbers of bone marrow cells (16–36 × 10⁶ cells per two femurs and two tibias). The only exceptions were mice 1* and 5**, which contained 3.0 × 10⁶ and 12 × 10⁶ bone marrow cells respectively.

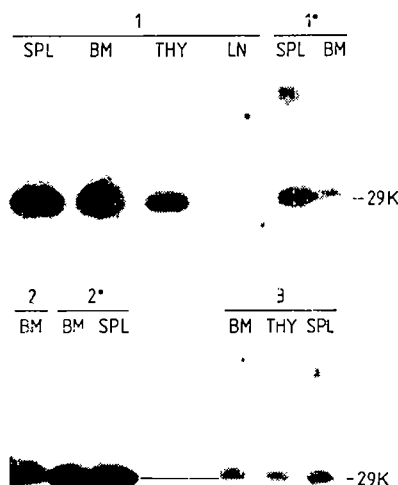


Fig. 3 Neomycin-phosphotransferase (NPT-II) activity in whole tissue extracts from long-term reconstituted mice. Preparation of tissue extracts and the *in situ* NPT-II assay are described in Fig. 2c. SPL, spleen; BM, bone marrow; THY, thymus; LN, lymph node; approximately five times less protein was loaded onto the LN lane. Animals 1 and 2 were reconstituted with preselected bone marrow cells, whereas mouse 3 received unselected cells. Mice 1* and 2* are recipients of bone marrow from mice 1 and 2 respectively.

(CFU-B), a subpopulation of mature B cells that are able to form colonies *in vitro*¹³, were grown in the presence of G418. Between 1 and 3% of the CFU-B found in the spleens of mice 2, 3, 4 and 5 were G418^r. Only 0.3% of those from mice 1* and 5* were able to grow in the presence of G418. Antibody secretion from cells in these G418^r colonies was demonstrated by their ability to form plaques as monitored by protein A plaque assay¹³.

In addition to growth of cells in G418, *neo* gene expression was monitored by assaying different tissue extracts from the reconstituted mice for NPT-II activity. Both myeloid and lymphoid organs of all long-term reconstituted mice tested expressed NPT-II activity (Fig. 3). As expected, organs from mice reconstituted with preselected cells (mice 1 and 2) contained higher enzyme levels than those from mice reconstituted with unselected cells (mouse 3). The presence of the *neo*-encoded protein in the different organs, together with the ability of the various precursors to grow in cultures containing G418, demonstrates unequivocally that the introduced *neo* gene is being expressed in all haematopoietic lineages.

Cell lineage analysis

The next series of experiments was designed to determine the number and the pattern of viral integration sites in the organs and in individual cell lineages of the reconstituted animals. Assuming that the site of integration of the provirus is random, information obtained from this analysis allows (1) estimation of the number of infected precursors that have participated in reconstitution of the different organs and (2) determination of the differentiation potential and longevity of these precursors.

Mice 1, 1*, 2, 2* and 3. Southern analysis of *Bam*HI-digested DNA from thymus, lymph node, spleen and bone marrow of mouse 1 showed three discrete fragments of 6.8, 9.4 and 16 kb (Fig. 4). These fragments were also present in bone marrow cells from the secondary recipient 1*. This finding suggests that a maximum of three precursors gave rise to the majority of *neo*-containing cells in the different organs of these mice. The intensity of the three bands compared with the intensity of a gene present as a single copy in all cells (hybridoma lanes in Fig. 4) indicates that a high proportion of the cells contain the *neo* provirus. From the presence of the same three bands in the different tissues, we conclude that the infected precursors were multipotent with the capacity to generate cells of the myeloid and lymphoid lineages. Finally, the presence of the same three integration sites in bone marrow of mouse 1*, indicates that this precursor (or precursors) could generate new cells capable of

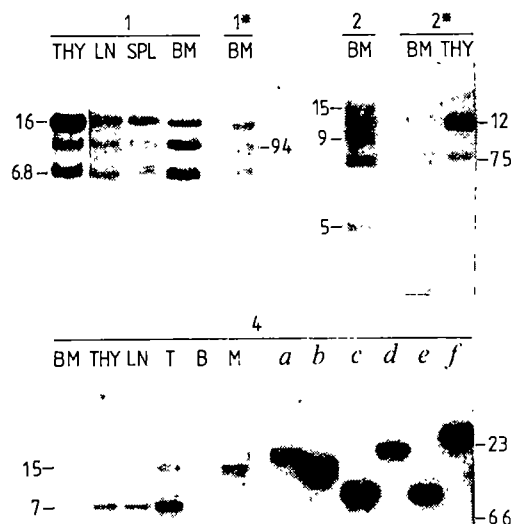


Fig. 4 Lineage analysis of three long-term reconstituted mice (1, 2 and 4). High-*M*_r DNA was isolated from various tissues, purified cell populations and from B-cell hybridomas and digested with *Bam*HI, which does not cut the proviral genome. Approximately 10 µg of digested DNA was fractionated on agarose gels and analysed by Southern blotting¹² using a *neo*-specific probe. 1* and 2* are recipients of bone marrow from mice 1 and 2. BM, bone marrow; THY, thymus; LN, lymph node; SPL, spleen; T, IL-2-dependent spleen cells; B, surface immunoglobulin-positive (sIg⁺) spleen cells; M, mast cells; a-f, B-cell hybridomas.

Methods. To derive IL-2-dependent cells, spleen cells were first stimulated with concanavalin A (Con A, 48 h) then cultured in medium containing either recombinant IL-2 (Roche) or supernatant from EL-4 cells²⁶ for 1–2 weeks. Over 98% of the cells used for DNA analysis were Thy-1.2-positive as determined by direct immunofluorescence using a labelled monoclonal antibody anti-Thy-1.2 and a fluorescence activated cell sorter (FACS II) for analysis. sIg⁺ cells were obtained following labelling of spleen cells with a fluorescein isothiocyanate-conjugated sheep anti-mouse antibody and sorting on a FACS II. Over 95% of the cells in the sorted population were sIg⁺. Mast cells^{27,28} were derived from bone marrow cells that were grown for more than 3 weeks in the presence of WEHI-3B(D⁺) conditioned medium and G418 (2 mg ml⁻¹). Most cells showed the characteristic granules for mast cells determined by astra blue²⁹ and toluidine blue staining³⁰. B-cell hybridomas were generated by fusion between the nonproducer F0 line and lipopolysaccharide (LPS)-stimulated B-cell blasts. Fusions were carried out 4 days after initiation of *in vitro* cultures with 50 µg ml⁻¹ lipopolysaccharide (LPS). G418 (1–2 mg ml⁻¹) was included on day 2 of these cultures. The LPS blasts were washed and fused according to methods described elsewhere³¹. Established hybridomas were selected for resistance to G418 before DNA analysis.

participating in the reconstitution of a second irradiated recipient. When DNA from the spleen of mouse 1 was digested with a second enzyme (*Hind*III), only three discrete bands were again detected, strongly suggesting that no more than three integration sites were present (data not shown).

DNA from bone marrow of mouse 2 contained at least five distinct integration sites (Fig. 4). On transfer of the bone marrow to mouse 2*, three of the hybridizing bands (5, 9 and 15 kb) were undetectable. Two of the bands found in the DNA of the primary bone marrow could also be found in the DNA from the thymus of mouse 2*. In addition, a fragment of higher relative molecular mass (*M*_r), which was not found in the primary mouse, was also present in the thymus DNA. Only the 12-kb band found in the bone marrow of mouse 2 was present in the DNA of bone marrow cells from mouse 2*. As this 12-kb fragment is also found in the thymus DNA of mouse 2*, it could represent a clone of cells derived from a primitive multipotent precursor. Again, the intensity of the bands in the bone marrow of mouse 2 and the thymus of mouse 2* strongly suggests that the majority of cells in these tissues contain the *neo* gene.

Mouse 3, reconstituted with unselected cells, contained a single integration site in the DNA from bone marrow, thymus, spleen and lymph node (data not shown). Only a small proportion of the cells in this animal had the *neo* gene, as determined by band intensity and colony growth (see Table 2). These

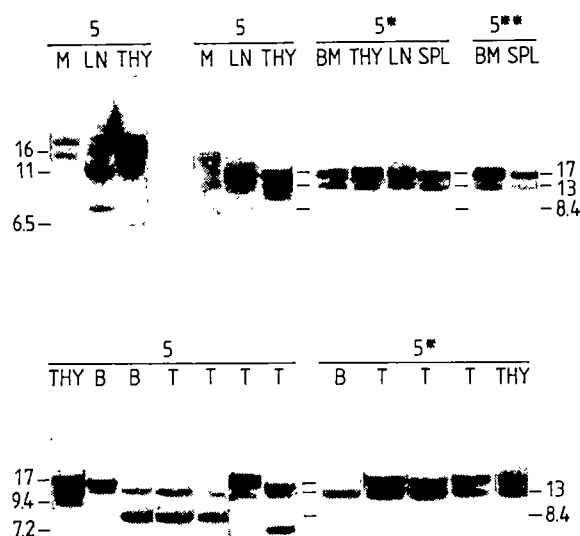


Fig. 5 Lineage analysis of mice 5, 5* and 5**. Southern analysis was carried out as described in Fig. 4 legend. DNA from all tissues, cell populations and hybridomas was digested with *Hind*III, an enzyme that does not cut the provirus genome. In addition, DNA from tissues of mouse 5 was also analysed following *Bam*HI digestion (see upper left-hand corner). Tissues and cell populations are designated as in Fig. 4 except for T, which refers to T-cell hybridomas.

Methods. T-cell hybridomas were generated by fusion between the thymoma BW5147 and spleen cells that had been stimulated with Con A in either the presence or absence of G418. Fusion was accomplished by standard techniques using polyethylene glycol 4000 (Merck) as a fusing agent. The hybridomas were first selected in hypoxanthine/aminopterin/thymidine medium and then in G418 (at least 1 mg ml^{-1} G418). From mouse 5, 8×10^6 cells stimulated without G418 and 3.5×10^6 cells stimulated in the presence of G418 were separately fused with a fivefold excess of BW5147 cells, then distributed into 24-well plates (3×10^5 cells per well). After several weeks all the wells from all the plates contained hybridomas; over 80% of the wells had cells that grew in G418. These G418^r cells were expanded and cloned by limiting dilution. From mouse 5*, 8×10^5 Con A blasts that had been stimulated in the presence of G418 were fused to 4×10^6 BW5147 cells and then seeded into two 24-well plates. This fusion generated six hybridomas, all of which were G418^r.

findings raised the question of whether the gene is present in cells of a number of different lineages or in only a single lineage that is found in all the organs examined. This problem was addressed in the analysis of mouse 4 by expanding cells of specific lineages *in vitro* in the presence or absence of G418 and including these in the DNA analysis.

Mouse 4. Interleukin-2 (IL-2)-dependent cells (T cells), and surface immunoglobulin-positive cells derived from the spleen and B-cell hybridomas were used to analyse the integration sites in the lymphoid lineages. Multilineage haematopoietic growth factor (MultiHGF/BPA/IL-3)¹⁴-dependent mast cells represented the myeloid lineages. A common band of 15 kb was found in the DNA from the thymus, lymph node, IL-2-dependent cells, mast cells and one of the six B-cell hybridomas (Fig. 4); this shows that cells from distinct lineages carry the same integration site and indicates that cells present in each of these populations arose from a common multipotent precursor. A similar interpretation is valid for the 7-kb band which is also present in the myeloid and lymphoid lineages.

Despite the fact that the organ DNAs showed two major integration sites, the cloned B-cell hybridomas exhibited five independent sites (Fig. 5). Only one of the fragments found in the B-cell hybridomas corresponded to the predominant 15-kb fragment found in the DNA from the tissues. Several bands found in other hybridomas corresponded to fainter bands that are visible in the DNA of the tissues, the IL-2-dependent cells and the mast cells. However, at least two could not be detected in the DNA of any cell lines or organs (Fig. 4*d, f*). With this analysis, it is not clear whether the unique fragments in the B-cell hybridomas are also present at low levels in the DNA of

the other lineages or whether they are restricted to the B-cell lineage.

To confirm that cells immortalized as hybridomas were of the B-cell lineage, DNA was next analysed for immunoglobulin heavy-chain gene rearrangements. Each hybridoma contained rearranged alleles and was found to be clonal in this analysis (data not shown).

Mice 5, 5* and 5.** The most detailed lineage analysis was carried out on mice reconstituted with N2-infected bone marrow cells. DNA from the organs, from mast cells and from B- and T-cell hybridomas of mouse 5 was analysed for the presence of unique viral integration sites. A similar analysis was performed with DNA from the organs, from T- and B-cell hybridomas of mouse 5*, and with DNA from the bone marrow and spleen of mouse 5**.

Two common integration sites of 17 and 13 kb were found in *Hind*III-digested DNA from all organs of the three mice (Fig. 5). The intensity of the hybridizing bands found in each of the tissues compared with the intensity of the bands found in the hybridomas suggests that a high proportion of cells are carrying the *neo* gene and are therefore of donor origin. This finding was confirmed by the presence of the T6 chromosome in more than 90% of metaphases from bone marrow cells of mice 5* and 5**. In addition to the two common bands, a fragment of lower M_r (8.4 kb) was found in mast-cell, lymph-node and thymus DNA of mouse 5. This fragment was present at low amounts in DNA from the thymus and spleen of mouse 5*, however, it was not detected in DNA from the spleen and bone marrow of the tertiary recipient (5**). Another integration site of 9.4 kb was found in the thymus of mouse 5, and in the spleen of mouse 5*. Finally, DNA from the mast cells and lymph node of mouse 5 contained fragments of higher M_r that were not detected in any other tissue. When DNA from mast cells, lymph node and thymus of mouse 5 was digested with *Bam*HI, at least four integration sites were found in each. Two of these sites (16 and 11 kb) appear to be common to all tissues and may correspond to the common sites present in the *Hind*III-digested DNA. The additional sites may be restricted to individual lineages.

The initial analysis of a limited number of B- and T-cell hybridomas from mice 5 and 5* is shown in the lower panel of Fig. 5. The two common bands present in the tissues are also found in one B-cell and two T-cell hybridomas from mouse 5 as well as in three T-cell hybridomas from mouse 5*. One of these T-cell hybridomas of mouse 5 contained, in addition, a third integration site of 7.2 kb, which was not detected in any of the tissues. Three other hybridomas of mouse 5 (one B-cell and two T-cell) also contained two sites that seem to be present in the tissues from this mouse. One B-cell hybridoma from mouse 5* had only one of the two common sites. This finding is difficult to interpret without further analysis. A more detailed study of the nature of the integration sites in the B- and T-cell hybridomas of these mice will be published elsewhere.

The presence of the same integration sites in mast cells, in B- and T-cell hybridomas, as well as in different tissues, is the most convincing demonstration to date of the existence of multipotent precursors able to generate myeloid and lymphoid cells. The persistence of integration sites in transfer from the primary to the secondary and tertiary recipients, suggests that the multipotent cell infected originally *in vitro*, exhibited substantial self-renewal capacity.

Discussion

The present report describes an efficient protocol for introducing retroviral vectors carrying a foreign gene into haematopoietic precursors of the mouse as well as the application of this method to cell lineage analysis. The protocol differs from methods used by others^{15,16} in several ways. First, bone marrow from 5-fluorouracil-treated rather than from normal mice was used for infection as it is enriched for primitive precursors¹⁷. Second, a preselection step was included in the protocol to obtain a cell population with a high frequency of infected precursors that were expressing the *neo* gene.

Using this protocol we have demonstrated that multipotent precursors common to the myeloid and lymphoid lineages can be infected with recombinant retroviruses. Furthermore, we have shown by the NPT-II assay and by growth of precursors in G418 that the introduced gene is expressed in all lineages of all reconstituted animals tested. Several other groups have recently described the transfer of genes to bone marrow cells using retroviral vectors^{15,16,18}. However, no-one has shown the infection of primitive multipotent precursors or expression of an introduced gene in distinct myeloid and lymphoid lineages.

From analysis of proviral integration sites we were able to enumerate the numbers of infected precursors which reconstituted each recipient. Through transfer of bone marrow from one recipient to the next, it was possible to estimate the longevity (self-renewal capacity) of such cells. Results from mice 1*, 2*, 5* and 5** indicated that some clones were maintained through transplantation, while others were lost (Figs 4, 5). Those clones that were lost might represent precursors at an intermediate stage of development while those that persisted could have been derived from the most primitive ones exhibiting self-renewal capacity. At least six multipotent precursors were identified in five different mice and four of these demonstrated self-renewal ability (Figs 4, 5). Of equal interest would be the identification of restricted precursors and in particular those restricted to the lymphoid lineages. Clones representing cells that might be restricted to the T-cell lineage are those marked by the 6.5-kb

fragment in the thymus and the 7.2-kb band in one T-cell hybridoma of mouse 5 (Fig. 5). Further analysis of viral integration sites from more reconstituted mice should identify such restricted precursors, thereby allowing a novel approach to studying cell lineage relationships.

In conclusion, we have shown that a foreign gene carried by a recombinant retrovirus can be efficiently introduced into haematopoietic precursors and that this gene is expressed in the progeny of these cells. We have also used the viral integration site as a clonal marker for identifying, enumerating and following the fate of haematopoietic precursors. Through analysis of unique viral integration sites, we have demonstrated unequivocally that cells with distinct lymphoid and myeloid phenotypes arose from a common precursor.

Since submission of this manuscript, Dick *et al.*¹⁹ have published a similar study on the introduction of a selectable gene into haematopoietic cells.

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Multiple sclerosis and human T-cell lymphotropic retroviruses

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A combination of different types of data suggests that some multiple sclerosis patients respond immunologically to, and have cerebrospinal T cells containing, a retrovirus that is related to, but distinct from, the three types of human T-cell lymphotropic viruses. The role of this virus in multiple sclerosis is uncertain.

ALTHOUGH viral infection has been postulated to play a part in multiple sclerosis (MS), a demyelinating disease of the central nervous system (CNS), there is no formal proof that any virus is involved in the aetiology of the disease. Since the discovery of the first human retroviruses¹, we have become interested in

their role as possible causes of various human diseases, including MS.

MS has some features that are characteristic of autoimmune disease. For example, it is associated with increases in absolute numbers of T cells in the cerebrospinal fluid (CSF)² and

occasionally by disturbances in the relative proportions of T-cell subpopulations during exacerbation or remission of the disease^{2,3}. A high level of oligoclonal immunoglobulin in MS CSF is a characteristic clinical finding⁴, and areas of demyelination in the brain (plaques) are usually associated with mononuclear infiltrates and often occur perivascularly⁵. Some characteristics of human retrovirus infection parallel features of autoimmune disease. The human T cell is the main target of infection of all known human retroviruses. T4-positive (helper-inducer) lymphocytes are transformed by human T-cell lymphotropic virus (HTLV)-I and -II⁶ and are killed by HTLV-III⁷, resulting in subpopulation imbalances. Infections with HTLV-I and HTLV-III have occasionally been associated with polyclonal B-cell activation and immunoglobulin production⁶. Moreover, T cells isolated from CSF of MS patients and maintained in culture closely resemble HTLV-transformed human T cells in their ability to express receptors for interleukin-2 (IL-2) for prolonged periods^{6,8}.

Recently, it was shown that HTLV-III can be detected in the brain⁹ and isolated from CSF and neural tissue of AIDS (acquired immune deficiency syndrome) patients¹⁰. Although the target cells await identification and may be of either neural or haematopoietic origin, the CNS pathology seen in MS involves cells of both lineages.

To study the possible involvement of human retroviruses in MS, we examined two sample populations. The first comes from Sweden, traditionally an area with a high incidence of MS; a recent large study reported an incidence of 5.3 per 100,000, with a female/male ratio of 1.5–1.6 and a peak age of onset of 20–39 yr¹¹. Although there is no evidence for Sweden, the uneven geographical distribution and the increasing prevalence of MS in neighbouring Finland have raised the possibility of a real increase in disease incidence¹². In western Norway, a threefold increase in prevalence from 20.1 to 59.8 per 100,000 was reported for the period 1963–83, reflecting a corresponding increase in incidence of new cases from 1.5 per 100,000 between 1953 and 1962 to 3.5 per 100,000 between 1968 and 1977 (ref. 13).

The second sample population comes from Key West, Florida, which has only recently become a high-risk area. Key West, an island 1.5 × 3.5 miles in size located 156 miles south-west of Miami, has a population of 26,450. Approximately 4,000 individuals are native-born. Although the first case of MS was diagnosed in Key West 57 years ago, it was not until 1981 that an unusual prevalence of the disease was suspected¹⁴.

We have examined the CSF and sera of MS patients from these two populations for the presence of antibodies reactive with antigens of the HTLV family. The availability of CSF T-cell lines from MS patients allowed us to screen for human retroviruses known to be T-lymphotropic by an assay that detects viral RNA in cells in which viral genes are expressed¹⁵.

Swedish patients

We examined paired samples of CSF and serum, in each case obtained on the same day, from 35 Swedish patients with definite MS, based on the criteria of McDonald and Halliday¹⁶. These patients were between 19 and 57 years old when the samples were obtained; 14 were male and 21 were female. The disease duration ranged from 10 days to 21 years (median 8 yr) from initial onset of symptoms. In 15 patients, optic neuritis marked clinical onset of the disease. Four patients were incapacitated, 11 had some symptoms and 20 were able to carry on normal activities. Samples from 15 patients were obtained during exacerbation, which had started between 5 days and 3 months previously. Fourteen other patients were in remission and six patients had progressive disease. One patient was receiving adrenocorticotrophic hormone (ACTH) therapy at the time of sampling and two patients had received ACTH 5 and 6 months before sampling, respectively.

Paired samples of CSF and serum were obtained on multiple occasions during exacerbation, remission and progressive disease from 3 of the 35 patients, who were aged 11, 24 and 34 yr at the onset of disease. This study was limited to three patients

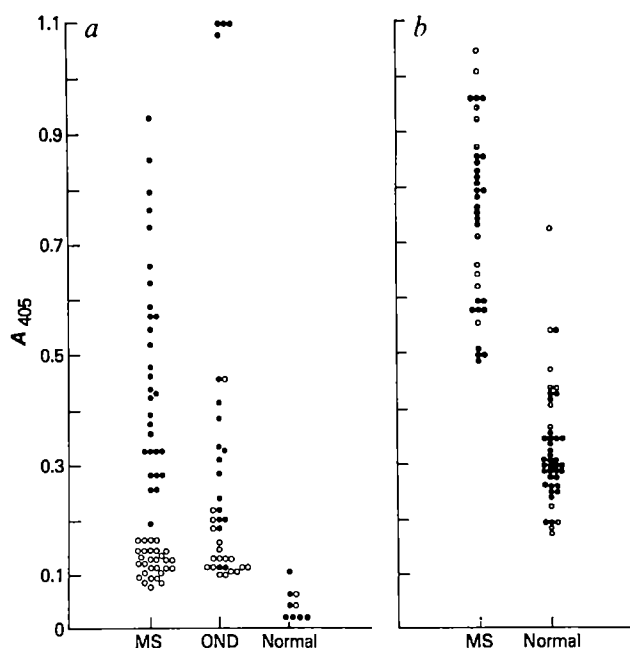


Fig. 1 *a*, Presence of antibodies reactive with HTLV-I p24 *gag* protein (●) and AMV p27 (○) in CSF from Swedish MS patients, patients with other neurological diseases (OND), and normal donors. All samples were tested simultaneously in the same laboratory. Data analysis by the Mann-Whitney *U*-test indicated that the MS CSF samples differed significantly from all OND CSF samples ($P=0.059$) and from OND samples taken from patients with an intact blood-brain barrier ($P=0.002$). *b*, Presence of antibodies reactive with HTLV-I p24 in sera of 35 Swedish MS patients and 43 normal Swedish donors. Serum samples from each group were diluted 1:150 in phosphate-buffered saline (PBS)/1% bovine serum albumin (BSA) and assayed for IgG antibody against HTLV-I p24 by ELISA as described for *a* below. Data analysis by the Mann-Whitney *U*-test indicated that the MS sera differed significantly from the normal sera ($P<0.001$). Results are the mean A_{405} for duplicate determinations.

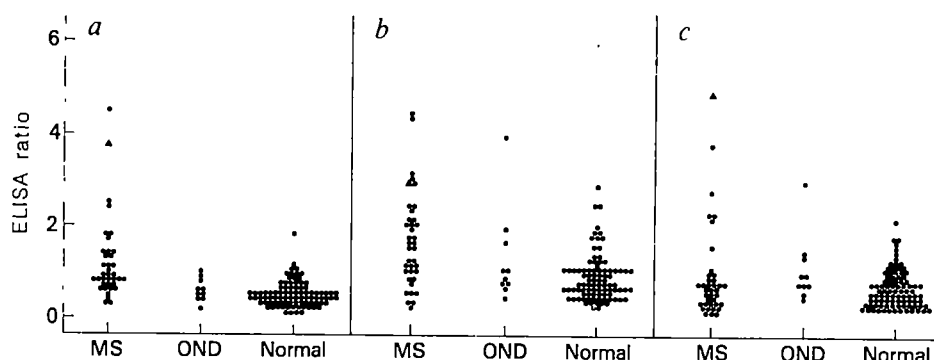
Methods. *a*, A single CSF sample from each patient was tested by ELISA using 2 $\mu\text{g ml}^{-1}$ p24 or p27 bound to Immulon II plates (Dynatech, Alexandria, Virginia) in carbonate-bicarbonate buffer, pH 9.9. Nonspecific binding was blocked by addition of 1% BSA in PBS. All patient CSF samples were diluted so that the concentration of IgG equalled that found in normal CSF, then applied to the plates. After 1 h at 23 °C, an optimal concentration of peroxidase-conjugated goat anti-human IgG (heavy and light chain; KPL, Gaithersburg, Maryland) was added for 1 h at 23 °C. After extensive washing, the plates were incubated with 2,2-azino-di-(3-ethylbenzthiazoline) sulphate and hydrogen peroxide and the plates read at 405 nm in a Titertek Multiscan (El Fab, Helsinki, Finland). Assay conditions were standardized using HTLV-I and HTLV-III-positive sera and normal control sera. Each assay included serum samples from an HTLV-I leukaemic patient (F0920) as a positive control and NIH HTLV-II-negative sera as a negative control.

because it involves multiple taps to obtain CSF samples in sufficient quantity to perform all assays. One of the patients received ACTH several times during the sampling (see legend to Fig. 3b).

In addition, a single CSF sample was obtained from each of 18 patients with neurological diseases other than MS (OND) and from 7 healthy subjects. As a control, serum samples were also obtained from 80 healthy Swedish hospital staff members and from blood donors.

Single CSF samples from each of 35 Swedish MS patients, 18 OND patients and 7 healthy subjects were evaluated for the presence of antibody reacting with the major core protein, p24, of HTLV-I¹⁷ by enzyme-linked immunosorbent assay (ELISA), as described in Fig. 1 legend. CSF from MS patients had higher levels of the anti-p24 antibody than CSF from normal individuals and from most of the OND patients (Fig. 1a). The three OND CSF samples that were notably reactive with p24

Fig. 2 Comparison of reactivity in ELISA of HTLV-I (a), -II (b) and -III (c) disrupted virions with serum antibodies of 35 MS patients, 10 OND patients and 80 normal blood donors (all Swedish)^{17,18} (●). All samples were tested simultaneously in the same laboratory. Additional tests for specificity of all sera were performed in two ways: (1) by competition assays using extracts of virus-producing cells, phytohaemagglutinin (PHA)-stimulated normal human peripheral blood lymphocytes, and fetal calf serum¹⁹; (2) by using HTLV-III type-specific antibody¹⁷. ▲, Sera were successfully competed in either assay by more than 50%.



were from a Guillain-Barré patient, a patient with meningitis of unknown origin and a patient with lymphocytic meningo-radulitis¹⁸. None of the MS CSF samples showed reactivity with the p27 of avian myeloblastosis virus (AMV) (Fig. 1a), which has low sequence homology with HTLV-I p24¹⁷, demonstrating the specificity of the antibodies for HTLV-I p24. Of the 18 OND samples tested, only the CSF from the meningo-radulitis patient was reactive with AMV p27.

ELISA analysis^{19,20} for HTLV-I p24 antibodies revealed significantly higher levels ($P < 0.005$) of these antibodies in sera from 35 MS patients (mean $A_{405} = 0.746 \pm 0.155$) than in 43 normal sera (mean $A_{405} = 0.325 \pm 0.102$) (Fig. 1b). For comparison, HTLV-I leukaemic serum F0920 routinely showed ELISA reactivity of $A_{405} 1.55-1.57$ in the same assay at the same serum dilution. To determine the specificity of the anti-p24 antibody in these sera, two assays were performed: one used a competitive radioimmunoassay (RIA) and the second a competitive ELISA. HTLV-I- and HTLV-III-positive sera were used to establish effective conditions to compete anti-p24 antibody with

disrupted virions of HTLV-I and of HTLV-III. In competitive-inhibition RIA and ELISA using serum samples from six MS patients, anti-p24 antibodies were completely inhibited by HTLV-I p24 and partially inhibited by disrupted HTLV-I and -III virions but not by AMV p27 (Table 1). In contrast, for serum from an HTLV-I leukaemic patient, anti-p24 antibody was completely inhibited by HTLV-I virions but not by HTLV-III virions.

The sera from the three OND patients whose CSF reacted with HTLV-I p24 also showed reactivity with HTLV-I p24. Whereas the reactivity of the case of lymphocytic meningo-radulitis¹⁸ could be considered nonspecific because the CSF reacted also with AMV p27, the case of Guillain-Barré may be linked with HTLV infection and it would be worthwhile to study more cases of the disease. The third OND case with meningitis of unknown origin could not be classified clinically.

Single serum samples from 35 MS patients, 10 patients with OND and 80 normal blood donors were evaluated by ELISA for reactivity with disrupted virions of HTLV-I, -II and -III²¹ (Fig. 2). The reactivity with HTLV-I of sera from MS patients (mean ratio 1.17) was significantly higher (Mann-Whitney U -test, $P < 0.003$) than that of OND sera (mean ratio 0.59) and of normal sera (mean ratio 0.45, $P < 0.001$). The reactivity of MS sera with HTLV-II (mean ratio 1.63) did not differ significantly from that of OND sera (mean ratio 1.24, $P < 0.08$) but did differ significantly from that of the normal sera (mean ratio 0.87, $P < 0.001$). Serum reactivity for HTLV-III showed no significant differences between all groups. Serum antibody in three MS patients was shown to be significantly inhibited by HTLV-I, -II or -III antigen or by specific antibody (see Fig. 2). No serum from OND or normal individuals was successfully competed with in these assays.

Serial CSF and serum samples from three MS patients, who were seen from the onset of their disease, were tested for antibody reactivity with HTLV-I p24 (Fig. 3). In the case of patient 01-12 (Fig. 3a), both the CSF and serum anti-p24 antibody levels fluctuated markedly between 1982 and 1984. A peak of CSF antibody in February 1983 was accompanied by elevation of the serum antibody, but the peaks of serum antibody observed in August 1983 and December 1984 were not accompanied by a rise in CSF antibodies. All antibody detected was of the IgG class; no IgM antibody was detected. The total IgG concentration in serum and CSF remained unchanged during the observation period (Fig. 3a); thus, the increase in specific antibody could not be explained by polyclonal increases in IgG levels.

In the second MS patient, 20-01 (Fig. 3b), p24 reactivity was detected in serum and CSF samples collected from 1977 to 1982; the two curves are parallel except from November 1981 onwards, when a rise in CSF antibody was accompanied by a decrease in serum antibody. Again, all antibody was IgG; no IgM was detected. The fluctuation in anti-p24 antibody concentration did not reflect changes in CSF and serum IgG levels, which remained relatively constant.

Table 1 Competitive inhibition for HTLV-I p24 antibodies in sera of Swedish MS patients

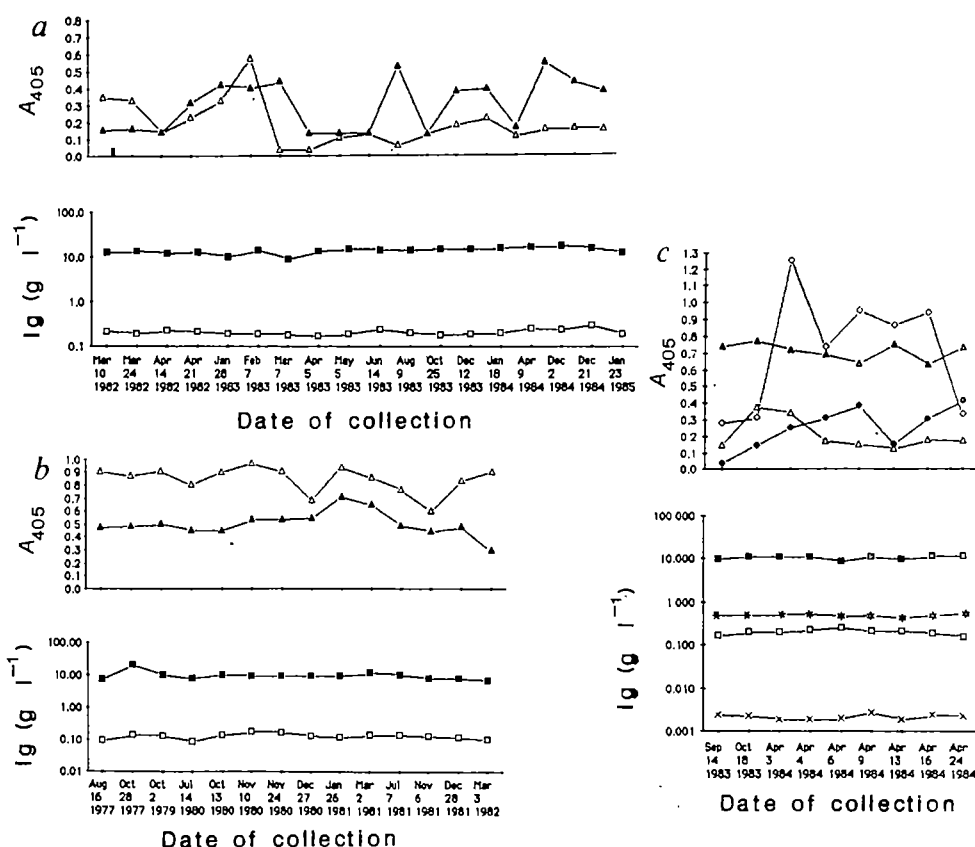
Patient	Competing antigen:			
	Disrupted virions HTLV-I	Disrupted virions HTLV-III	HTLV-I p24	AMV p27
13-10*	46	44	86	6
01-12*	29	30	83	4
3380†	58	51	98	NT
2268†	40	31	83	-8
2167†	61	29	89	NT
1738†	29	15	86	NT
F0920†‡	90	28	94	NT

* Patients' sera were diluted 1:25 and incubated overnight on plates precoated with goat anti-human IgG as a 'catcher' antibody. Inhibition of HTLV-I p24 antibody was determined by reacting various concentrations of disrupted virions of HTLV-I and -III, AMV p27 and HTLV-I p24 with wells containing patients' sera for 3 h at 37 °C. After washing, binding of HTLV-I p24 was assessed by incubating all cells with 0.25 μ g 125 I-labelled HTLV-I p24 (1×10^5 c.p.m. per well). Inhibition is expressed as $[(^{125}\text{I-p24 c.p.m. bound with no competing antigen} - ^{125}\text{I-p24 c.p.m. bound with competing antigen}) / \text{total } ^{125}\text{I-p24 c.p.m.}] \times 100$. Values are the percentage inhibition of $^{125}\text{I-p24}$ binding by 0.5 μ g of competing antigen.

† Specificity of antibodies against HTLV-I p24 in four MS sera was determined by competitive inhibition in an ELISA with soluble HTLV-I p24, disrupted virions of HTLV-I and -III and AMV p27. Equal volumes of patients' sera (1:100 dilution) were incubated with soluble antigen (0.7 μ g ml^{-1}) for 30 min then applied to plates precoated with HTLV-I p24 as described in Fig. 1 legend. Per cent inhibition was calculated as follows: $[(A_{405}$ of sera without soluble competitor $- A_{405}$ of sera with competitor) / A_{405} competition] $\times 100$. NT, not tested.

‡ Serum from an HTLV-I leukaemic patient was diluted 1:500 and inhibitors used at 0.35 μ g ml^{-1} .

Fig. 3 Antibody reactivity with HTLV-I p24 in serial samples of CSF and sera obtained from Swedish MS patients during their disease, compared with concentrations of IgG and IgM in the same CSF and sera. All samples were tested simultaneously in the same laboratory. *a*, Patient 01-12: onset of MS in September 1977 at age 34 yr. The course of the disease was characterized by frequent, acute and short exacerbations, particularly during the study period from 1982 to 1984. The patient received no ACTH or steroid treatment. *b*, Patient 20-01: onset of MS in November 1976 at age 24 yr. The course of the disease was characterized by exacerbations from its onset to 1983, with slow progression of the disease thereafter. The patient received two ACTH treatments in December 1980 and December 1981. *c*, Patient 13-10: onset of MS in March 1976 at age 11 yr with exacerbations in 1979, 1980, April and September 1983, and April 1984, with complete recovery between exacerbations. Patient treated with ACTH only during April 1983, 1 yr before the exacerbation in April 1984. Δ , IgG antibody to HTLV-I p24 in CSF; \blacktriangle , IgG antibody to HTLV-I p24 in sera; \square , \blacksquare : concentration of IgG in CSF and sera, respectively; \times , $*$: concentration of IgM in CSF and sera, respectively.



Δ , IgG antibody to HTLV-I p24 in CSF; \blacktriangle , IgG antibody to HTLV-I p24 in sera; \square , \blacksquare : concentration of IgG in CSF and sera, respectively; \times , $*$: concentration of IgM in CSF and sera, respectively.

Methods. The assay for HTLV-I p24 antibodies was as described in Fig. 1 legend. The immunoglobulin concentrations were determined by an electroimmunoassay described elsewhere²². Serum samples were diluted for the ELISA assay to achieve approximately the same immunoglobulin concentration as found in the CSF samples in order to compare the relative amounts of specific antibody. The immunoglobulin concentrations plotted are therefore those used in the assay.

In the third patient sampled, 13-10 (Fig. 3c), it was possible to follow the anti-p24 antibody levels over several months before and during an acute clinical exacerbation in April 1984. A dramatic rise in CSF anti-p24 antibody of the IgM class was detected between 14 September 1983 and 3 April 1984, accompanied by a lesser but still significant rise in CSF IgG antibody to p24. During April 1984, the CSF IgG antibody decreased to its original value. The CSF IgM antibody levels remained high until 13 April 1984, after which they returned to the levels observed before exacerbation. The concentration of IgM serum antibody fluctuated, whereas that of the IgG serum antibody remained relatively high during the observation period. None of these changes can be explained by concomitant changes in CSF or serum immunoglobulin concentrations, which remained relatively stable during the observation period.

Intrathecal production of immunoglobulin

IgG and albumin concentrations in CSF and serum from the Swedish MS patients were determined²² to obtain the IgG-albumin index (CSF IgG \times serum albumin / serum IgG \times CSF albumin), an indicator of intrathecal immunoglobulin production in CSF²³. Values above 0.66 indicate intrathecal immunoglobulin production in patients with an intact blood-brain barrier²³. For the three patients who were repeatedly sampled, the indices ranged from 0.81 to 6.82, with only two samples showing values <1.00 . For the MS patients in Fig. 1, values ranged from 0.50 to 2.16, with only four values less than 0.66. Thus, a portion of the CSF IgG of these patients is synthesized in the CNS and does not originate in the blood.

The ratio of CSF albumin to serum albumin is taken as an indicator of the integrity of the blood-brain barrier²⁴; this ratio

was within the normal range for all MS patients, indicating an intact blood-brain barrier. However, the three OND patients with high levels of CSF antibody reactive with HTLV-I p24 (Fig. 1a) had ratios of 0.033, 0.020 and 0.011, compared with 0.0074 for normal donors, indicating that the blood-brain barrier was not intact, permitting leakage of serum proteins into the CSF.

Patients from Key West, Florida

At the time of assessment on 1 September 1983, 37 patients with MS were identified in Key West¹⁴. Only one was not Caucasian, and there were two pairs of siblings with MS and two patients with MS-afflicted siblings living elsewhere. The diagnosis of MS was again based on the criteria of McDonald and Halliday¹⁶. Of the 37 patients, 17 were available to us for this study. Five were born in Key West; of the other 12, 5 had moved there at ages of 5–18 yr and 7 at age 22–39 yr. The onset of MS preceded this study by 1–5 yr in 12 patients, by 9 yr in one patient, and by 14, 16, 19 and 20 yr in the remaining patients. Three patients had never received steroid therapy, 13 had received steroid or ACTH therapy (no later than 1983) and one patient was being treated with Immuran at the time of study.

Paired samples of CSF and serum were available from 9 of the 17 patients, and serum samples were available from the remaining 8 patients. A second serum sample obtained 4 weeks later was available from 9 of the 17 patients. Seventeen close contacts of the patients (spouse, parent, sibling) and 17 other individuals also donated blood for this study.

Table 2 shows the number of sera from MS patients and from their contacts that were reactive with HTLV-I p24 and disrupted virions of HTLV-I, -II and -III at one point in time. Seven serum

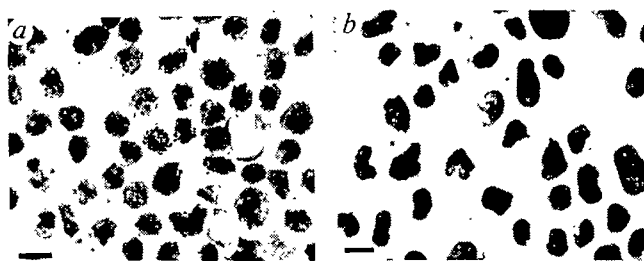


Fig. 4 Detection of cells expressing RNA with homology to HTLV-I in cultures derived from CSF of MS patients. *a*, Representative field of cultured cells obtained originally from CSF of native-born Key West MS patient no. 4 and hybridized with an HTLV-I 3' probe. This patient showed onset of MS in 1982 at age 21 yr. The disease followed a relapsing-remitting course with accumulated moderate neurological deficits attributed to spinal cord involvement; there was major manifestation of mild to moderate ataxia and spastic paraparesis. Labelled cells were present at a frequency of 0.01%. *b*, Representative field of cultured cells obtained from CSF of a healthy Swedish subject and hybridized with the HTLV-I 3' probe, as described for *a*. No labelled cells present. Pictures of representative fields of cultured cells obtained from two other Key West MS patients and one Swedish patient (see text) were similar to *a*. Scale bar = 10 μ m.

Methods. Freshly isolated CSF cells were co-cultured in cluster plates (Costar) with autologous γ -irradiated peripheral blood mononuclear cells in complete medium containing purified PHA (PHA-P, 1 μ g ml⁻¹; Burroughs-Wellcome) for 3 days. Cell concentrations were adjusted to 2×10^5 ml⁻¹ in complete growth media with 50 ng ml⁻¹ recombinant IL-2 (Sandoz, Vienna, Austria). Cells were maintained in logarithmic growth by periodic supplementation with fresh media containing IL-2 and by stimulation with PHA-P. All cell lines were maintained in the same laboratory before hybridization. *In situ* hybridization was carried out using ³⁵S-labelled RNA probe specific for the 3' region of HTLV-I¹⁵. The RNA probe was transcribed from a full-length genomic clone of HTLV-I and averaged 1–2 kilobases long. Probe was hybridized at $1\text{--}2 \times 10^8$ d.p.m. ml⁻¹ in relaxed conditions (45 °C) and autoradiographed for 4–8 days. All cell lines were hybridized in the same conditions in the same laboratory, and cells were examined using a double-blind code.

samples from MS patients were reactive with HTLV-I, four with HTLV-II and one with HTLV-III; three sera also reacted with HTLV-I p24. Serum of one patient reacted with all three HTLV types, whereas serum of another patient reacted with only HTLV-I and -II. Of the 17 MS sera, 10 were reactive with an HTLV antigen at least once. Of the 17 donors who had intimate contact with the MS patients, two showed serum reactivity with HTLV-III: one of the sera, obtained from a homosexual brother of one of the MS patients, was HTLV-III-seropositive; his serum reactivity might reflect true exposure to the HTLV-III agent of AIDS rather than a cross-reactivity. The second donor was a male not known to be in any HTLV high-risk group. None of the sera from 17 people employed at the hospital where most of the MS cases were treated showed reactivity with HTLV-I, -II or -III.

Of nine CSF samples available from these MS patients, only one sample was reactive with HTLV antigens, specifically with HTLV-I p24. Of the eight negative samples, seven were from patients whose sera were reactive with HTLV antigens.

HTLV-I-related nucleotide sequences

In situ hybridization was used to examine cultured CSF cells obtained from five Key West MS patients, three Swedish MS patients and two healthy Swedish subjects for the presence of HTLV-specific RNA by methods described previously (ref. 15 and unpublished results with M. Feinberg), using specific molecularly cloned HTLV-I and HTLV-III probes. So far, three of the CSF cultures derived from Key West MS patients (who had suffered from MS for 3, 4 and 21 yr) and one CSF culture derived from a Swedish MS case (who had suffered from the disease for 1 month) have shown the presence of labelled cells

following hybridization under low-stringency conditions (45 °C instead of 50 °C) with the HTLV-I 3' RNA probe, which contains predominantly long terminal repeat sequences. These positive cells exhibited an average of 50 grains per cell (Fig. 4*a*) and were observed at a frequency of from 0.001 to 0.01% of the 5×10^5 cells generally examined. In contrast, hybridization of the same HTLV-I probe under identical conditions to cultured CSF cells from two normal individuals consistently failed to reveal labelled cells (Fig. 4*b*); similarly, there were negative results with normal peripheral blood cells, bone marrow and other cell types. Hybridization of an HTLV-I probe specific for the *gag* coding region to CSF cells from two Key West MS patients also revealed rare labelled cells. Thus, cells in four MS CSF cultures contain RNA that hybridizes with HTLV-I under low-stringency conditions. In several experiments in which hybridization was carried out under standard stringency conditions (50 °C), no labelled cells were observed in CSF from one Key West MS patient. Although difficult to quantitate because of the low number of positive cells observed after hybridization at 45 °C, the lack of detectable label under conditions of higher stringency suggests that the RNA detected shares only partial homology with the HTLV-I genome. No hybridization was detected in the same MS CSF cultures using an HTLV-III 3' RNA probe under reduced stringency, indicating a lack of RNA homologous to this virus in the cultured cells.

Discussion

The data presented here indicate the presence of antibodies reactive with HTLV antigens in CSF of Swedish MS patients at concentrations significantly higher than those in CSF samples from Swedish OND patients and from healthy donors. The three OND patients with high CSF levels of anti-HTLV antibodies were shown to have an impaired blood-brain barrier, which suggests that the antibodies were derived from serum. The CSF from seven healthy subjects with intact blood-brain barriers showed no reactivity. Antibodies reactive with HTLV-I p24 antigen (Fig. 1*b*) and with disrupted virions of HTLV-I and -II (Fig. 2) were at higher levels in sera of Swedish MS patients than in sera from OND patients (Fig. 2) or from normal healthy subjects (Figs 1*b*, 2). Perhaps even more significant is the fact that of 17 sera collected from Key West MS patients at various times, 10 reacted at one time or another with an HTLV antigen, whereas reactivity was observed in only 2 of 17 sera obtained from close contacts of the patients (one of whom was a homosexual with serum antibody against HTLV-III) and in none of 17 sera from hospital staff workers from the same area.

One cannot exclude the possibility that, as in the case of measles antibodies in CSF of MS patients²⁵, the HTLV antibodies in CSF represent only a small fraction of the immunoglobulin produced intrathecally by MS patients. Unlike measles antibodies²⁶, however, the level of HTLV antibodies fluctuated in the CSF of MS patients from whom serial CSF samples were available. In one case studied (Fig. 3*c*), a sharp rise of these antibodies accompanied an exacerbation of the clinical disease. It is important, therefore, to emphasize that the antibody reactivity identified in some MS sera or CSF was detected at some periods but not at others.

No uniform response to one particular type of HTLV was observed; this was especially obvious in the Key West MS patients, in whom serum reactivity with any of the three types of HTLV varied among patients. In two cases, the same serum samples reacted with more than one type of HTLV (Table 2). The HTLV-I anti-p24 reactivity in six Swedish MS sera was comparably inhibited by HTLV-I and HTLV-III disrupted virions (Table 1), which may reflect recognition of an epitope(s) on HTLV-I p24 which is shared with other types of HTLV²⁷. The fact that none of the Swedish MS CSF samples reacted with AMV p27, and that 10 HTLV-reactive sera from Key West patients did not react with disrupted virions of visna virus or gibbon ape leukaemia virus indicates that the antibody response of the MS patients is directed specifically towards one or more HTLV-related proteins. But as our data suggest that it is not

Table 2 HTLV-reactivity of sera from Key West patients, intimate contacts and hospital workers

Clinical status	No. of patients	No. of sera reacting with:				No. of positive cases/total*
		HTLV-I p24	HTLV-I	Disrupted virions of HTLV-II	HTLV-III	
MS	17	3	7	4	1	10†/17
Contacts	17	1	0	0	1	2‡/17
Hospital workers	17	0	0	0	0	0/17

Reactivity was determined by ELISA; the results were all confirmed by competition with either antigens of or specific antibodies to HTLV-I, -II and -III¹⁷⁻¹⁹. All samples were tested simultaneously in the same laboratory. The contacts and hospital workers groups were each compared with MS patients using Yates' χ^2 analysis: $\chi^2 = 5.44$ ($P = 0.020$) for contact group; $\chi^2 = 9.67$ ($P = 0.002$) for hospital workers.

* The positive results in the MS patients are transient; they are found at only a specific point in time in any given patient.

† Serum of one patient reacted with all three types of HTLV; serum of another patient reacted with types I and II, and serum of one patient reacted only with HTLV-I p24.

‡ One case was a homosexual brother of an MS patient.

directed exclusively at any of the three known HTLV types, it is difficult to apply the rigid criteria established for evaluation of serological results in diseases linked unquestionably with either HTLV-I (leukaemia, lymphoma) or HTLV-III (AIDS). Indeed, interpretation of serological results in the present study is more arbitrary and the detection of HTLV antibodies should in no way be considered of diagnostic value for MS.

The detection of RNA homologous to HTLV-I in CSF cells of four MS cases whose sera reacted with HTLV-I antigen (Table 2) indicates that a human retroviral genome is being expressed and suggests the possibility that a virus is present. The low frequency of hybridizing cells is similar to the situation observed in primary samples of T cells from HTLV-III-infected patients (AIDS and AIDS-related complex, ARC), where 0.01% or less of cells express RNA¹⁵. The *in vitro* cultivation of T cells infected with HTLV-III results in increased expression of RNA and protein; the actual percentage of cells expressing virus after *in vitro* cultivation is probably related to the frequency of virus-infected cells in the original sample. *In situ* hybridization studies with another retrovirus, visna virus, indicated that fewer than 1% of cells expressing low amounts of viral RNA were found in the tissue samples taken from infected sheep.^{28,29}

As the presence of anti-HTLV antibodies in the CSF of MS patients suggests an antigenic stimulation from the CNS, it seems reasonable to search in the CNS for the presence of the appropriate antigen. The only evidence that the human brain is susceptible to HTLV infection is the detection of HTLV-III in brains of children and adults with AIDS encephalopathy¹⁰. In diseases caused by HTLV, the target for primary virus infection is the peripheral T cell. If the same is true in MS, infected peripheral T cells might transport the virus to the CNS, cells of which might be permissive for viral replication. Damage to myelin-producing glial cells could result directly from viral infection. Whereas no evidence exists for the presence of virions in MS brain, it should be stressed that a specific search for the presence of human retrovirus or its components in brain cells has not yet been made.

The presence of CSF cells that hybridize with the HTLV-I probe in MS patients but not in healthy subjects may suggest that T cells present in CSF become infected during the disease. This hypothesis is strengthened by the fact that some T cells obtained from CSF of MS patients share characteristics with HTLV-transformed T cells. For example, like HTLV-transformed T cells⁶, one clone from CSF cells of a Swedish MS patient had a low, but significant, number of cells with lobular or multiple nuclei⁸. In addition, most mitogen-induced MS CSF T-cell lines consisted predominantly of OKT3,4-positive cells⁸, as did cells isolated from HTLV-associated T-cell malignancies and cord blood lymphocytes transformed *in vitro* with HTLV-I⁶. Finally, most MS CSF T-cell lines grow to saturation densities of $\geq 2 \times 10^6 \text{ ml}^{-1}$ (ref. 8), like the HTLV-transformed lymphocytes⁶, and grow for extended periods of time in exogenous IL-2 only⁸. The exception is the one clone mentioned above, which is IL-2 receptor-positive but neither produces nor depends

on IL-2 for growth; in this regard, these cells resemble both cord blood and bone marrow-derived lymphocytes transformed by HTLV-I^{30,31}.

If an HTLV-related retrovirus is at all involved in the aetiology of MS, how does one account for three of our findings: (1) the serological detection of anti-HTLV antibodies in less than 70% of the patients; (2) the consistently low level of serum antibody responses in those sera showing some reactivity; and (3) some variation in specificity of the antibody response to HTLV-I, -II or -III? Assuming MS is a disease caused by one agent, the first observation might be explained by the fluctuation in antibody levels during the disease, so that antibodies might not be detected in a single CSF or serum specimen. Alternatively, all MS patients might be infected but only a proportion might respond with the production of antibodies that cross-react with HTLV, or the agent might consistently fail to induce detectable amounts of antibodies. In fact, HTLV-III infection does not induce an antibody response in all infected subjects although the proportion is around 4% (refs 32, 33). Regarding point (2), the low titre of antibodies could be due to transient and/or low-level expression of the putative virus, or to its cross-reactivity with the known HTLV types being very slight. Finally, MS may consist of several subtypes with different aetiologies and the infectious HTLV-related agent may be a factor in only some subtypes. Our data indicate that the agent detected in this study is not HTLV-I, -II or -III. A related but distinct retrovirus might be expected to cross-react weakly with one or more antigenic and genomic determinants of the known HTLVs, just as a weak cross-reactivity with HTLV-I probes led to the discovery of HTLV-II^{34,35}.

The variation in the reactivity of the antibodies with HTLV-I, -II or -III may be explained if the epitopes recognized by these antibodies partially cross-react with the known HTLV types. A novel HTLV related to HTLV-I would be expected to react with HTLV-II antigens in view of the substantial homology between these two retroviruses^{34,35}. Furthermore, some antibodies might be more reactive with HTLV-II proteins than with HTLV-I if the new HTLV had greater homology with HTLV-II than with HTLV-I. However, homology cannot be used to explain the two serum samples from MS patients which were reactive with both HTLV-III and HTLV-I or HTLV-II (Fig. 2, Table 2), as the genome of HTLV-III differs substantially from the other two types. This could conceivably be explained by an HTLV with the properties of a recombinant of the two major types, with variation in expression of specific viral antigen(s) among different patients and even in the course of the disease in a given patient.

The incubation period for HTLV-I and -II is 5–30 yr^{34,36}. A similar incubation period for the putative HTLV-related retrovirus would be consistent with the notion of exposure in early adolescence to any infectious agent that might be involved in the aetiology of MS³⁷.

We have discussed our data as though they indicate the presence and involvement of a novel HTLV-related virus in MS.

If that is the case, our attempts to isolate the virus should eventually be successful. For now, the data are suggestive, but not conclusive.

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LETTERS TO NATURE

A blue stellar population in the H I bridge between the two Magellanic Clouds

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Radio observations have shown¹ that a common H I cloud surrounds the Magellanic Cloud system. However, no stellar link between the two galaxies has previously been found. The furthest known stellar extension eastward of the Small Magellanic Cloud (SMC) is the blue association of stars found by Kunkel², which lie close to the extreme tip of the 'wing' of the SMC. The SMC wing was discovered by Shapley³, who described it as "a large cloud of faint stars extending eastward from the SMC toward the LMC [Large Magellanic Cloud]". More recently, Westerlund⁴ described the brighter ($V < 20.0$) stellar population of the wing as being a mixture of extreme Population I, OB associations, H II regions and both blue and yellow supergiants. The rest of the Magellanic system shows a strong correlation between H I density and the presence of stellar condensations, and this has led to speculation that stellar associations may be present in the H I extension of the optical wing (the H I bridge indicated in Fig. 1) and also independently in regions of high H I density in the Magellanic stream⁵. Deep H α photography⁶ shows weak diffuse emission between the Magellanic Clouds, supporting the possible existence of an optical counterpart to the H I bridge, but star counts near the eastern tip of the wing⁷ failed to reveal any link and indeed corroborated earlier work on the structure of the wing⁸. We now report the discovery of several hundred blue main-sequence stars in an area reaching 9° east of the SMC, coinciding with the H I ridge extending from the wing. These stars lie midway between the two Clouds and are more than 2 kpc further east than what was believed to be the eastern tip of the SMC wing. The apparent magnitudes of the brightest main-sequence stars suggest an age of ~100 Myr, similar to ages of associations in the wing.

The discovery² of stellar aggregates at the extreme eastern tip of the wing prompted us to investigate a large area to the east of the wing. For this purpose we obtained a pair of UK Schmidt plates (IIIaJ+GG385 and IIIaF+OG590) centred on RA 02 h 37 min, dec. -73° 43'. The plates were scanned and processed by the Automatic Plate Measuring (APM) facility at Cambridge and calibrated from a photoelectric sequence (W.E.K., M.J.I. and S.D., in preparation). Magnitudes (R) and colours ($J-R$) were obtained for over 120,000 stellar objects in a 6°×6° area. This number corresponds to a magnitude limit of $m_J \sim 21$ and $m_R \sim 20$.

The area covered by the plates and its relationship to the Magellanic system are shown in Fig. 1. Three levels of integrated H I column densities are shown for the LMC⁴ and the SMC⁹, corresponding to levels of 4, 8 and $\geq 12 \times 10^{20}$ H I atoms cm⁻². The optical centres¹⁰ of both galaxies are represented by dots.

The colour-magnitude diagram of the 120,000 stars reproduced in Fig. 2 reveals a well-defined blue main sequence, extending upward to $m_R \sim 15$, and well separated from the expected foreground galactic stars. Multicolour photometry¹¹

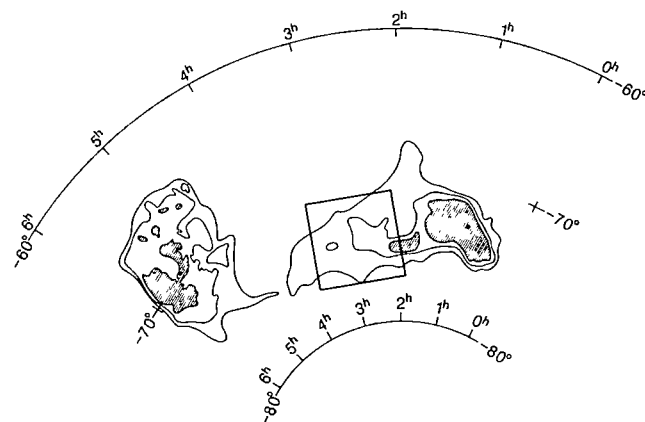


Fig. 1 The field investigated (square) relative to the Magellanic Clouds represented here by three levels of H I column density: 4, 8 and $\geq 12 \times 10^{20}$ atoms cm⁻². The dots represent the optical centres of the two galaxies.

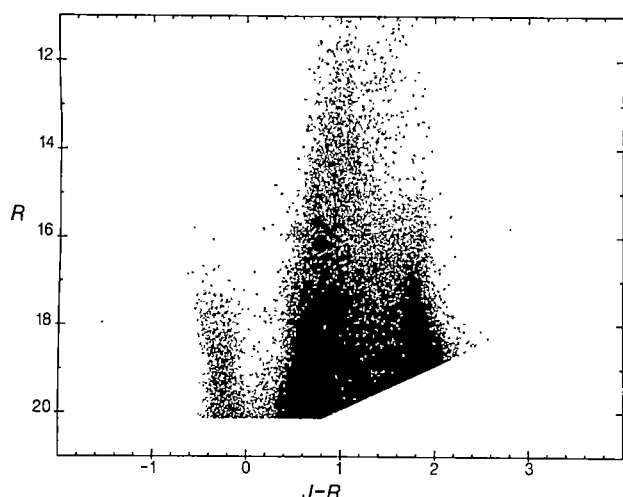


Fig. 2 A stellar colour-magnitude diagram in photographic J (B_J) and R of the $6^\circ \times 6^\circ$ of sky investigated. A Zero Age Main Sequence (ZAMS) is clearly discernible (dashed line) as are the foreground galactic halo (yellow $J-R \sim 0.8$) and disk (red $J-R \sim 1.9$) stars. Some 4,000 stars are in the region blueward of $J-R=0$.

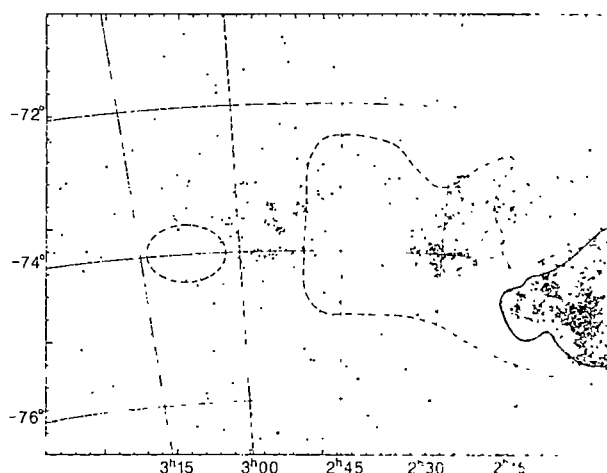


Fig. 3 The spatial distribution of the stars with colour $J-R < 0$. The tip of the wing (see ref. 8) is outlined (solid line) together with the H I isophote corresponding to 8×10^{20} atoms cm^{-2} (dashed line).

of Cepheids in the system has shown that the colour excess toward the tip of the wing is small, $E(B-V) \leq 0.05$, indicating that these blue stars have magnitudes and colours consistent with a sequence located at roughly the same distance as the Magellanic Clouds. Figure 3 gives the spatial distribution of the $\sim 4,000$ main-sequence stars with $J-R < 0$. These stars represent around 3% of the total stellar population of the 36 deg^2 area. The tip⁸ of the wing and the H I isophotes are outlined. As expected, most of the blue stars, occur in the extreme tip of the wing, seen on the right (Fig. 3). The concentration at RA 02 h 30 min coincides with the previously known stellar aggregate². We see here for the first time a large area of blue stars lying to the north of the extreme tip, and the group of blue stars at RA 02 h 52 min of angular extent $\sim 1^\circ$. This latter group is 9° from the optical centre of the SMC and 12.5° from the LMC, corresponding to distances of 10.6 and 12.5 kpc, respectively, which are well outside the radius¹⁰ of both clouds. Furthermore, Fig. 3 clearly reveals an overall optical counterpart to the H I bridge, although some of the blue stars are apparently in regions of low H I density.

Colour-magnitude diagrams for all the areas possessing a marked excess of blue stars have been analysed separately. Apart from number density of images, all the regions have almost identical blue main sequences (within ± 0.1), confirming that these blue stars indeed belong to the Magellanic Clouds. By statistically removing an equivalent foreground comparison colour-magnitude diagram, it is also possible to search the region with colours $J-R > 0$ for red giants and horizontal branch stars. Preliminary results indicate that the combined number of Magellanic red giants and horizontal branch stars is less than 10% of the number of blue main-sequence stars in the selected areas. Furthermore, for stars with $J-R > 0$, no clumpiness or overall stellar density gradient is discernible across the field, as observed⁷ for the halo region of the SMC. Even limiting the search to those stars having the magnitude and colour of the horizontal branch stars produces no definitive non-uniformity of images. This is not too surprising as these stars form less than 1% of the total population at these magnitudes and colours and are therefore swamped by the foreground component.

As a further check, number density maps of the whole $6^\circ \times 6^\circ$ area were constructed. These reveal a distinct excess of images only in the regions where the extra blue stars are found, confirming that it is the blue main-sequence stars that dominate the bridge population. Also, the noise in the number density map indicates that although the extra stars are visible with hindsight on deep photographs, two-colour photometry is

needed to identify them unambiguously. All this evidence strongly suggests that we are looking outside what is known as the SMC. There is no doubt from the individual colour-magnitude diagrams that the main-sequence stars are at a similar distance to the Magellanic Clouds. Photometry of Cepheids¹² throughout the SMC strongly suggests that the wing is pointing towards us, thus becoming progressively closer as it goes further east. The nearest well-studied¹³ cluster, Lindsay 113, lies in the wing 4.5° east of the optical centre of the SMC at a distance of 58 kpc. This is midway between the accepted distance¹² for the SMC and LMC of 62 and 54 kpc, respectively. We will adopt a preliminary distance of 58 kpc for the Magellanic bridge region, keeping in mind that recent observations¹⁴ have suggested that the distances of both Magellanic Clouds may require a major revision.

At this distance the brightest main-sequence stars are at $V \sim 15$ or $M_V \sim -4$. Adopting an age calibration¹⁵ of the Magellanic Cloud clusters leads to an age estimate of less than 100 Myr for the new blue stellar associations. This is comparable with the ages¹⁶ of clusters and associations in the wing. A study leading to a more accurate knowledge of the chemical composition, age and distance (and hence origin) of the stars in the bridge region is now under way (W.E.K., M.J.I. and S.D., in preparation). Preliminary results from the photographic study indicate that the blue stars in the bridge region have a common origin with similar blue stars in the SMC wing and that they are located midway between the two Clouds.

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C₆₀: Buckminsterfullerene

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During experiments aimed at understanding the mechanisms by which long-chain carbon molecules are formed in interstellar space and circumstellar shells¹, graphite has been vaporized by laser irradiation, producing a remarkably stable cluster consisting of 60 carbon atoms. Concerning the question of what kind of 60-carbon atom structure might give rise to a superstable species, we suggest a truncated icosahedron, a polygon with 60 vertices and 32 faces, 12 of which are pentagonal and 20 hexagonal. This object is commonly encountered as the football shown in Fig. 1. The C₆₀ molecule which results when a carbon atom is placed at each vertex of this structure has all valences satisfied by two single bonds and one double bond, has many resonance structures, and appears to be aromatic.

The technique used to produce and detect this unusual molecule involves the vaporization of carbon species from the surface of a solid disk of graphite into a high-density helium flow, using a focused pulsed laser. The vaporization laser was the second harmonic of Q-switched Nd:YAG producing pulse energies of ~30 mJ. The resulting carbon clusters were expanded in a supersonic molecular beam, photoionized using an excimer laser, and detected by time-of-flight mass spectrometry. The vaporization chamber is shown in Fig. 2. In the experiment the pulsed valve was opened first and then the vaporization laser was fired after a precisely controlled delay. Carbon species were vaporized into the helium stream, cooled and partially equilibrated in the expansion, and travelled in the resulting molecular beam to the ionization region. The clusters were ionized by direct one-photon excitation with a carefully synchronized excimer laser pulse. The apparatus has been fully described previously²⁻⁵.

The vaporization of carbon has been studied previously in a very similar apparatus⁶. In that work clusters of up to 190 carbon atoms were observed and it was noted that for clusters of more than 40 atoms, only those containing an even number of atoms were observed. In the mass spectra displayed in ref. 6, the C₆₀ peak is the largest for cluster sizes of >40 atoms, but it is not completely dominant. We have recently re-examined this system and found that under certain clustering conditions the C₆₀ peak can be made about 40 times larger than neighbouring clusters.

Figure 3 shows a series of cluster distributions resulting from variations in the vaporization conditions evolving from a cluster distribution similar to that observed in ref. 3, to one in which C₆₀ is totally dominant. In Fig. 3c, where the firing of the vaporization laser was delayed until most of the He pulse had passed, a roughly gaussian distribution of large, even-numbered clusters with 38–120 atoms resulted. The C₆₀ peak was largest but not dominant. In Fig. 3b, the vaporization laser was fired at the time of maximum helium density; the C₆₀ peak grew into a feature perhaps five times stronger than its neighbours, with the exception of C₇₀. In Fig. 3a, the conditions were similar to those in Fig. 3b but in addition the integrating cup depicted in Fig. 2 was added to increase the time between vaporization and expansion. The resulting cluster distribution is completely dominated by C₆₀, in fact more than 50% of the total large cluster abundance is accounted for by C₆₀; the C₇₀ peak has diminished in relative intensity compared with C₆₀, but remains rather prominent, accounting for ~5% of the large cluster population.

Our rationalization of these results is that in the laser vaporization, fragments are torn from the surface as pieces of the planar

Fig. 1 A football (in the United States, a soccerball) on Texas grass. The C₆₀ molecule featured in this letter is suggested to have the truncated icosahedral structure formed by replacing each vertex on the seams of such a ball by a carbon atom.



graphite fused six-membered ring structure. We believe that the distribution in Fig. 3c is fairly representative of the nascent distribution of larger ring fragments. When these hot ring clusters are left in contact with high-density helium, the clusters equilibrate by two- and three-body collisions towards the most stable species, which appears to be a unique cluster containing 60 atoms.

When one thinks in terms of the many fused-ring isomers with unsatisfied valences at the edges that would naturally arise from a graphite fragmentation, this result seems impossible: there is not much to choose between such isomers in terms of stability. If one tries to shift to a tetrahedral diamond structure, the entire surface of the cluster will be covered with unsatisfied valences. Thus a search was made for some other plausible structure which would satisfy all sp² valences. Only a spheroidal structure appears likely to satisfy this criterion, and thus Buckminster Fuller's studies were consulted (see, for example, ref. 7). An unusually beautiful (and probably unique) choice is the truncated icosahedron depicted in Fig. 1. As mentioned above, all valences are satisfied with this structure, and the molecule appears to be aromatic. The structure has the symmetry of the icosahedral group. The inner and outer surfaces are covered with a sea of π electrons. The diameter of this C₆₀ molecule is ~7 Å, providing an inner cavity which appears to be capable of holding a variety of atoms⁸.

Assuming that our somewhat speculative structure is correct, there are a number of important ramifications arising from the existence of such a species. Because of its stability when formed under the most violent conditions, it may be widely distributed in the Universe. For example, it may be a major constituent of circumstellar shells with high carbon content. It is a feasible constituent of interstellar dust and a possible major site for

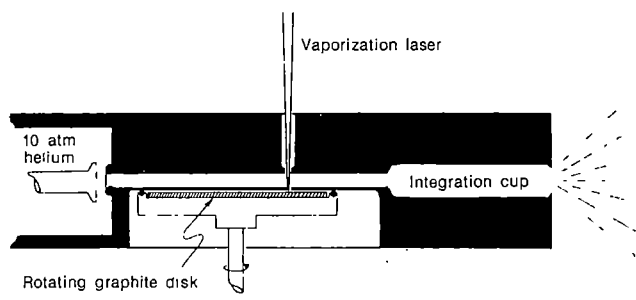


Fig. 2 Schematic diagram of the pulsed supersonic nozzle used to generate carbon cluster beams. The integrating cup can be removed at the indicated line. The vaporization laser beam (30–40 mJ at 532 nm in a 5-ns pulse) is focused through the nozzle, striking a graphite disk which is rotated slowly to produce a smooth vaporization surface. The pulsed nozzle passes high-density helium over this vaporization zone. This helium carrier gas provides the thermalizing collisions necessary to cool, react and cluster the species in the vaporized graphite plasma, and the wind necessary to carry the cluster products through the remainder of the nozzle. Free expansion of this cluster-laden gas at the end of the nozzle forms a supersonic beam which is probed 1.3 m downstream with a time-of-flight mass spectrometer.

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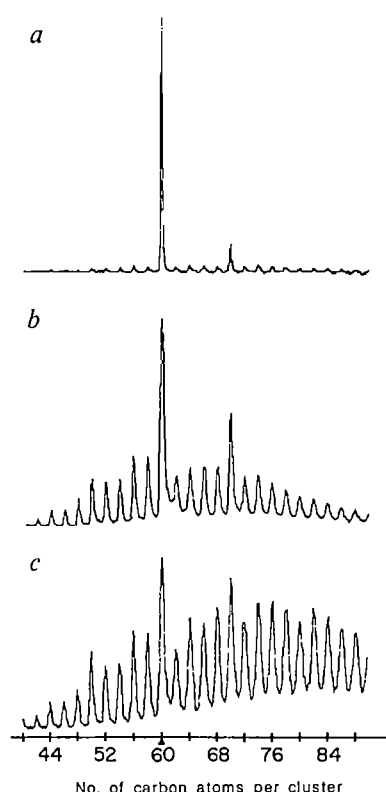


Fig. 3 Time-of-flight mass spectra of carbon clusters prepared by laser vaporization of graphite and cooled in a supersonic beam. Ionization was effected by direct one-photon excitation with an ArF excimer laser (6.4 eV, 1 mJ cm⁻²). The three spectra shown differ in the extent of helium collisions occurring in the supersonic nozzle. In *c*, the effective helium density over the graphite target was less than 10 torr—the observed cluster distribution here is believed to be due simply to pieces of the graphite sheet ejected in the primary vaporization process. The spectrum in *b* was obtained when roughly 760 torr helium was present over the graphite target at the time of laser vaporization. The enhancement of C₆₀ and C₇₀ is believed to be due to gas-phase reactions at these higher clustering conditions. The spectrum in *a* was obtained by maximizing these cluster thermalization and cluster-cluster reactions in the 'integration cup' shown in Fig. 2. The concentration of cluster species in the especially stable C₆₀ form is the prime experimental observation of this study.

surface-catalysed chemical processes which lead to the formation of interstellar molecules. Even more speculatively, C₆₀ or a derivative might be the carrier of the diffuse interstellar lines⁹.

If a large-scale synthetic route to this C₆₀ species can be found, the chemical and practical value of the substance may prove extremely high. One can readily conceive of C₆₀ derivatives of many kinds—such as C₆₀ transition metal compounds, for example, C₆₀Fe or halogenated species like C₆₀F₆₀ which might be a super-lubricant. We also have evidence that an atom (such as lanthanum⁸ and oxygen¹) can be placed in the interior, producing molecules which may exhibit unusual properties. For example, the chemical shift in the NMR of the central atom should be remarkable because of the ring currents. If stable in macroscopic, condensed phases, this C₆₀ species would provide a topologically novel aromatic nucleus for new branches of organic and inorganic chemistry. Finally, this especially stable and symmetrical carbon structure provides a possible catalyst and/or intermediate to be considered in modelling prebiotic chemistry.

We are disturbed at the number of letters and syllables in the rather fanciful but highly appropriate name we have chosen in the title to refer to this C₆₀ species. For such a unique and centrally important molecular structure, a more concise name would be useful. A number of alternatives come to mind (for

example, ballene, spherene, soccerene, carbosoccer), but we prefer to let this issue of nomenclature be settled by consensus.

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High-resolution solid-state NMR of quadrupolar nuclei

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Quadrupolar nuclei are the most abundant nuclear magnetic resonance (NMR)-receptive nuclei in the Earth's crust, and in many amorphous materials of technological interest (such as zeolite catalysts, ceramics and alloys), and have thus been intensively studied¹⁻⁷. Of particular interest is the ability to resolve and quantitate the various types of sites present in a given material. Here we present a very simple, yet we believe powerful, approach towards the resolution of chemically non-equivalent sites in solids, which combines a conventional high-field spin-echo NMR method with the resolution enhancement of the 'quadrupole shift' approach^{4,7}. We demonstrate its application to the complete resolution of both the (1/2, 3/2) and (3/2, 5/2) transitions of the ²⁷Al nuclei in a mixture of potassium and ammonium alums (KAl(SO₄)₂·12H₂O and NH₄Al(SO₄)₂·12H₂O).

The major problem in spin-echo studies of most quadrupolar nuclei in solids is that a series of echoes is usually obtained^{8,9}. Solomon first reported⁸ the refocusing of first-order quadrupolar interactions using two radio-frequency pulses of the same phase, and Butterworth⁹ was able to differentiate quadrupolar from purely magnetic interactions in various alloys. However, this approach is not particularly useful for minerals and other non-metallic materials, where large magnetic inhomogeneities are absent.

Fortunately, however, Bonera and Galimberti¹⁰ and Weisman and Bennett¹¹ showed that considerable enhancement of the 'desired' 2 τ echo could be achieved by introducing a 90° phase-shift between the two radio-frequency pulses. Moreover, Weisman and Bennett showed (for nuclear spin $I = 5/2$) for the special case of a 90°- τ -45° pulse sequence that the amplitude of the 2 τ echo (central plus quadrupolar contribution) was maximized, while the 'allowed' 3/2 τ plus 3 τ echo amplitude was minimized¹¹. In practice, the 2 τ echo is often the only one observed¹¹, and we show here that the Fourier transform of the echo can yield an essentially undistorted (1/2, -1/2; 1/2, 3/2; 3/2, 5/2) powder spectrum.

Figure 1*a* and *b* show the ²³Na and ²⁷Al spin-echo spectra of NaNO₃ and KAl(SO₄)₂·12H₂O, obtained at magnetic field strengths of 8.45 and 3.52 T, respectively, the response of the potassium alum being obtained in the presence of ¹H dipolar

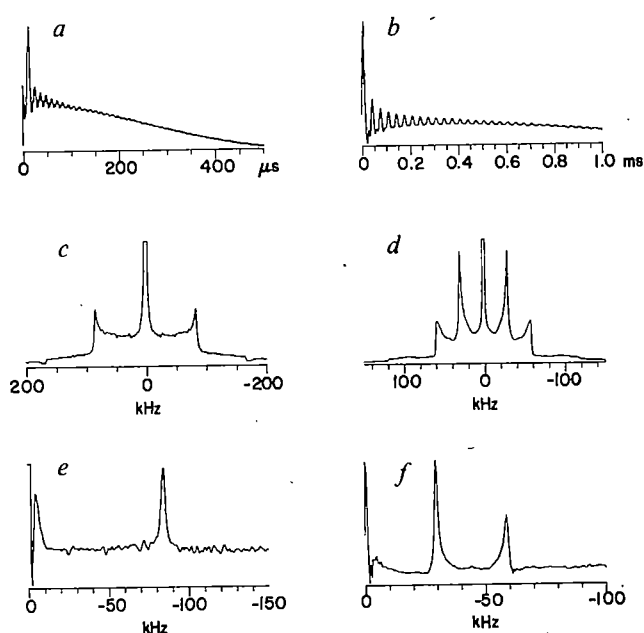


Fig. 1 Quadrupole-echo NMR spectra of ^{23}Na in NaNO_3 and ^{27}Al in $\text{KAl}(\text{SO}_4)_2 \cdot 12\text{H}_2\text{O}$, obtained at magnetic field strengths of 8.45 and 3.52 T, respectively. *a*, ^{23}Na spin-echo from NaNO_3 ; *b*, ^{27}Al spin-echo from $\text{KAl}(\text{SO}_4)_2 \cdot 12\text{H}_2\text{O}$; *c*, Fourier transform of half-echo in *a* showing central ($1/2, -1/2$) and satellite ($1/2, 3/2$) transitions; *d*, Fourier transform of half-echo in *b* showing central ($1/2, -1/2$) and satellite ($1/2, 3/2$; $3/2, 5/2$) transitions; *e*, de-Paked version of *c* showing the 90° edge, corresponding to $e^2qQ/h \sim 339$ kHz, η assumed ~ 0 ; *f*, de-Paked version of *d* showing the 90° edges of the ($1/2, 3/2$) and ($3/2, 5/2$) transitions, corresponding to $e^2qQ/h \sim 395$ kHz, η assumed ~ 0 .

decoupling (at 150 MHz). No subsidiary echoes were observed for ^{27}Al (and none were expected for ^{23}Na), even though, with our present instrumentation, we were unable to use pulses much stronger than the overall spectral breadth. Nevertheless, Fourier transformation of both responses yielded conventional frequency domain powder-pattern spectra (Fig. 1*c, d*) in which the 90° edges of the $1/2, 3/2$ (Na, Al) and $3/2, 5/2$ (Al) transitions were well resolved.

To provide a more convenient 'high-resolution' presentation, we have applied the 'de-Pakeing' algorithm developed by Bloom *et al.*^{12,13} to the non-integral spin spectra of Fig. 1*c, d*, and show the results in Fig. 1*e* and *f*. For both systems, there were no appreciable second-order effects, and asymmetry parameters $\eta \sim 0$, so narrow-line spectra corresponding to nuclear quadrupole coupling constants, $e^2qQ/h = 339$ kHz (NaNO_3) and 395 kHz ($\text{KAl}(\text{SO}_4)_2 \cdot 12\text{H}_2\text{O}$) were obtained, in good agreement with previous single crystal determinations¹⁴⁻¹⁷.

To illustrate further the usefulness of the method, which can be implemented on a conventional high-field NMR instrument, Fig. 2*a-c* gives the spin-echo, spin-echo Fourier transform powder pattern and de-Paked lineshape for an equimolar mixture of potassium and ammonium alums, again obtained in the presence of ^1H dipolar decoupling. The K^+ and NH_4^+ alum signals have their expected 1:1 intensity ratios. In both cases, the potassium and ammonium alum signals are fully resolved, the outer satellite transitions being slightly more resolved than the inner ones (even though their component linewidths are greater). Further line-narrowing may be possible in other cases by application of homonuclear line-narrowing techniques (when e^2qQ/h values are very small). The quadrupole coupling constants (395 kHz and 441 kHz for potassium alum and ammonium alum, respectively) and asymmetry parameters ($\eta = 0$ for both) show excellent agreement with the single crystal NMR data¹⁵⁻¹⁷, which give $e^2qQ/h = 400$ kHz for potassium alum and 446 kHz for ammonium alum, and $\eta = 0$ for both (since the point symmetry of the ^{27}Al is $\bar{3}$ in both structures).

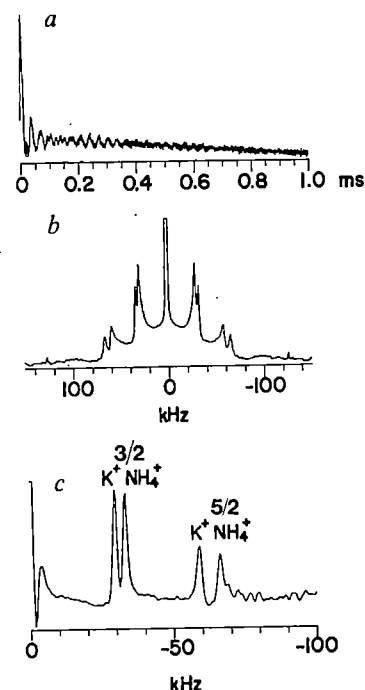


Fig. 2 Quadrupole-echo NMR spectra of ^{27}Al in a 1:1 molar mixture of $\text{KAl}(\text{SO}_4)_2 \cdot 12\text{H}_2\text{O}$ and $\text{NH}_4\text{Al}(\text{SO}_4)_2 \cdot 12\text{H}_2\text{O}$, obtained at a magnetic field strength of 3.52 T, in the presence of ^1H dipolar decoupling. *a*, ^{27}Al spin-echo; *b*, Fourier transform of half-echo showing central ($1/2, -1/2$) and overlapping satellite ($1/2, 3/2$; $3/2, 5/2$) transitions for the K^+ and NH_4^+ alums. *c*, de-Paked version of *b* showing complete resolution of both K^+ and NH_4^+ alums in both satellite transitions, corresponding to $e^2qQ/h \sim 395$, 441 kHz, η assumed ~ 0 .

Overall, we believe that the above results indicate considerable promise for high-field NMR techniques in the differentiation of signals in powder samples from quadrupolar nuclei of identical chemical shifts. The technique should be applicable to systems having larger e^2qQ/h values by using high-power wide-band transmitters at higher field (using smaller sample, low- Q probes). Such an application was limited in the present case by the use of pulses having ~ 0.5 - μs rise and fall times, but should not be limited to systems having long spin-lattice relaxation times. It can be readily implemented (at least for small e^2qQ/h values) on existing conventional Fourier transform NMR instruments, and could thus be extremely useful in the structural analysis of a variety of amorphous solids of interest in the earth, materials and biological sciences. For all nuclei, operation with very wide-bore magnets (to house the very high-voltage capacitors needed for short pulse widths) at the highest magnetic field strengths possible (to minimize second-order quadrupole interactions and 'ringing' effects) will, however, be essential in order to maximize the usefulness of this approach.

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A hydrated aluminophosphate with both 4.8² and 6³ sheets in the 4-connected framework

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Porous aluminosilicates of the zeolite type have become important industrially as ion-exchangers, molecular sieves and catalysts^{1,2}. Topologically, the tetrahedral centres of the oxygen tetrahedra lie at the nodes of various 4-connected three-dimensional nets. Four-connected porous frameworks (AlPO₄) composed of alternating AlO₄ and PO₄ tetrahedra are the basis of a new family of synthetic molecular sieves, which may become important industrially because they lack the strong ion-exchange and catalytic properties of the zeolites³. Aluminophosphates display a wide range of crystal structures because Al can be coordinated to either four, five or six oxygen atoms, some of which may belong to either hydroxyl or water species^{3–12}. As part of a systematic study of the crystal chemistry of aluminophosphates, we have found that the framework of synthetic-phase H3 (ref. 13) (AlPO₄·1.5 H₂O) contains PO₄ tetrahedra alternating between AlO₄ tetrahedra and AlO₄(H₂O)₂ octahedra. As reported here, the 4-connected three-dimensional net formed by linking adjacent Al and P atoms is a new type containing 6.6.6 and 4.8.8. two-dimensional nets joined by up-down linkages. This coexistence of two types of two-dimensional nets in a three-dimensional net suggests that the principle of parsimony should not be adopted too strictly in attempts to invent new nets of potential relevance to molecular sieve technology.

Crystals of a hydrated aluminium phosphate were synthesized by S. T. Wilson, B. M. Lok and E. M. Flanigen of Union Carbide Corporation. The X-ray powder diffraction pattern of the crystals matches that in Fig. 2 of ref. 13. Full details of the X-ray structure determination will be published elsewhere¹². Cell dimensions and atomic coordinates (Table 1) were refined by the least-squares method. Refinement was straightforward except for the H atoms. The positions of H-1 and H-2 were constrained to lie at 0.85 Å from O-9; the same was true for O-10, H-3 and H-4. Whereas these two water oxygens are bonded to Al-2, the last oxygen O-11, is not part of the framework and its position is weakly constrained. Because hydrogen atoms could not be located even from a constrained refinement, it is assumed that O-11 is part of a loosely bonded water molecule.

Figure 1 is a stereo plot showing the positions of all located atoms. Each P atom is tetrahedrally coordinated, and shares an oxygen atom with four adjacent Al atoms. Similarly, each Al-1 atom shares an oxygen atom with one P-2 and three P-1 atoms. Each Al-2 atom, however, is bonded to two water molecules as well as four framework oxygens. Such an octahedral complex, AlO₄(H₂O)₂, is also found in metavariscite⁵. The atomic positions in Table 1 correspond to AlPO₄·1.5 H₂O instead of the AlPO₄·1.67 H₂O listed by d'Yvoire¹³. The complex dehydration and rehydration properties of H₃ could be interpreted in terms of easy loss of the water molecule at position O-11, conversion of the octahedral complex AlO₄(OH)₂ to a tetrahedral complex AlO₄ by loss of two water molecules at a higher temperature, and adsorption of water by the dehydration product on cooling in a humid atmosphere; however, further structure determinations are needed to clarify this.

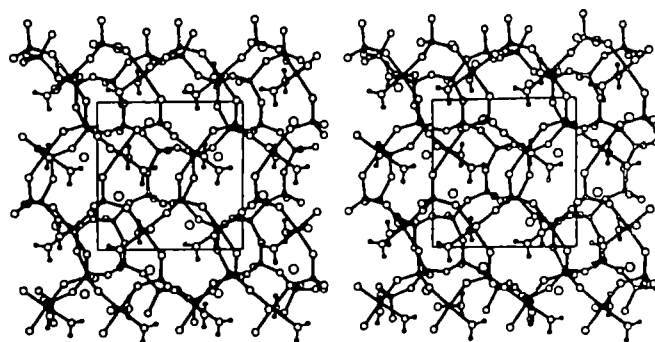


Fig. 1 Stereo plot of H3 structure type, projected down the *a* axis with *c* pointing upwards and *b* sideways. Small open and solid circles at line intersections represent Al and P atoms, respectively. Large open circles represent oxygen atoms. Pairs of H atoms (small dots) are shown for each water molecule attached to an Al atom, but not for the loosely bonded water molecule.

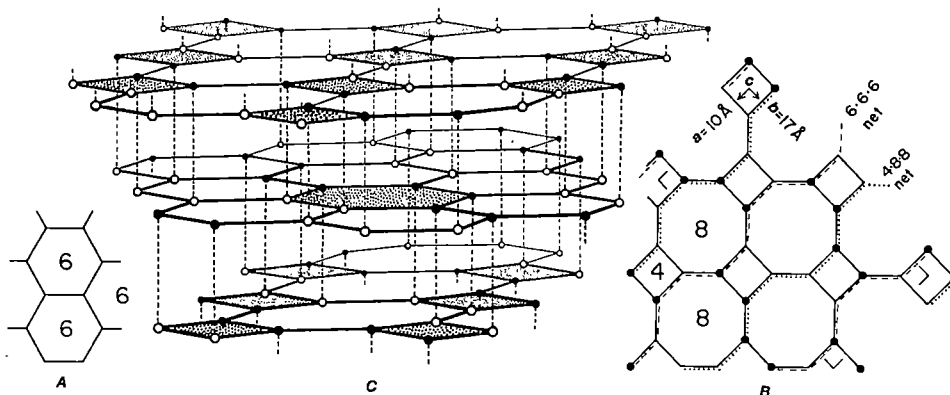
When the water molecules are ignored, the structure can be regarded as a 4-connected tetrahedral framework which can be compared with the growing list of theoretical and three-dimensional nets (ref. 14 and refs therein; also refs 15, 16). The H3 net is novel, and it is interesting because it is based on up-down linkages between both 6³ and 4.8² nets. A portion of a 3-connected 6.6.6 two-dimensional net is shown in plan view in Fig. 2A; each node is connected by three edges, each of which is part of a circuit of six edges. The continuous lines of Fig. 2B represent part of a 3-connected 4.8.8 two-dimensional net. An infinite number of three-dimensional nets can be obtained by stacking horizontal 6.6.6 two-dimensional nets in a vertical pile and connecting adjacent nodes by a vertical linkage¹⁷. Because only one linkage can be added to each 3-connected node, there are two choices for each node. The simplest three-dimensional nets have been enumerated, and some are found in crystalline materials¹⁷. Similar enumeration has been done for 4.8.8 nets connected by up-down linkages¹⁸. The H3 tetrahedral framework is shown in clinographic perspective in Fig. 2C. A 6.6.6 net (centre) is connected to 4.8.8 nets above and below it by vertical linkages (dashed lines). Each 3-connected node marked by an open circle is joined to a node above it (solid circle) so that each node becomes 4-connected. Regular alternation of 6.6.6 and 4.8.8 nets in the vertical stack generates an infinite 4-connected three-dimensional 3D net. The 6.6.6 and

Table 1 The atomic positions ($\times 10^5$) and mean-square displacements (\AA^2) of AlPO₄-H3

Atom	<i>x/a</i>	<i>y/b</i>	<i>z/c</i>	U_{eq} or U_{iso}
P(1)	44,769 (4)	34,071 (8)	13,498 (9)	84 (2)
P(2)	29,050 (4)	6,565 (8)	33,879 (9)	78 (2)
Al(1)	44,808 (4)	8,470 (9)	32,419 (10)	89 (3)
Al(2)	28,207 (5)	33,224 (9)	14,654 (10)	79 (3)
O(1)	37,433 (10)	37,652 (22)	17,006 (24)	146 (7)
O(2)	46,047 (12)	16,616 (22)	48,037 (24)	174 (7)
O(3)	49,520 (10)	44,967 (21)	19,709 (24)	163 (7)
O(4)	46,889 (11)	20,038 (20)	19,416 (23)	134 (7)
O(5)	27,397 (10)	5,819 (20)	49,128 (22)	114 (7)
O(6)	36,522 (10)	1,895 (22)	31,355 (24)	160 (7)
O(7)	28,329 (11)	21,582 (20)	29,532 (23)	133 (7)
O(8)	25,855 (10)	47,583 (20)	25,919 (22)	112 (7)
O(9)	18,446 (12)	28,311 (27)	11,931 (31)	220 (8)
O(10)	30,512 (12)	17,298 (23)	3,398 (26)	155 (8)
O(11)	11,929 (14)	14,398 (33)	36,268 (37)	568 (11)
H(1)	15,752 (12)	29,879 (27)	5,232 (31)	197 (37)
H(2)	16,096 (12)	25,744 (27)	18,827 (31)	150 (25)
H(3)	28,123 (12)	10,248 (23)	5,406 (26)	66 (16)
H(4)	30,108 (12)	19,016 (23)	-5,101 (26)	54 (17)

a = 19.352(1), *b* = 0.727(1), *c* = 9.762(1) Å; orthorhombic, Pbcn. Room temperature. CuK α . Crystal 40 \times 40 \times 220 μ m, elongated *c*. 1,439 unique diffraction intensities. Anisotropic thermal parameters. *R* = 0.04. $U_{iso}(\times 10^3)$ is given for hydrogen atoms, and $U_{eq}[(10^3/3)\sum_i U_{ij}a_i^*a_j^*(a_i, a_j)]$ is given for the other atoms. Number in parentheses represent standard error.

Fig. 2 Topological drawings. **A**, Plan view of a portion of a 6.6.6 3-connected two-dimensional net. **B**, Plan view of a portion of a 4.8.8 3-connected two-dimensional net (continuous lines). The corner marks outline the *c*-axis projection of the unit cell for the H3 structure type idealized to the regular geometry for up and down linkages from a 4.8.8 two-dimensional net (up, node marked by solid circle; down, unmarked node). Dotted and broken lines show, respectively, side views of the 4.8.8 and 6.6.6 nets in *C*. **C**, Perspective drawing of the up-down linkages (dashed lines) between alternating 4.8.8 and 6.6.6 two-dimensional nets. Twelve 4-rings and one 6-ring are stippled to strengthen the perspective.



4.8.8 nets are distorted from the ideal shapes when the linkages are vertical. In the H3 structure (Fig. 1), the nets are considerably crinkled to allow for bonding to the water molecules, but may become more regular on complete dehydration.

The H3 structure can also be described in terms of up-down linkages between a vertical stack of 4.8.8 nets. Figure 2B shows a plan view of a three-dimensional net obtained when adjacent groups of four linkages (solid circles) alternate with four downward linkages (unmarked nodes). The unit cell for the most regular geometry and an edge length of 3.1 Å is orthorhombic, with $a = 17$ Å, $b = 10$ Å, $c = 8.5$ Å, space group Abmm, 32 nodes (Z_c) per cell, 16 nodes (Z_i) in the asymmetric unit, circuit symbol $4^3 6^2 8$. (Z_c is the number of atoms in the crystallographic unit cell and Z_i is the number of atoms in the smallest unit cell.) This net was not enumerated previously¹⁸ because its a repeat of 17 Å is longer than the upper limit of 15 Å selected by Smith and Rinaldi¹⁹; it is now denoted as net 24a. Crinkling of the framework in the H3 structure changes the above cell to $a = 19.35$ Å, $b = 9.73$ Å, $c = 9.76$ Å, Pbc_a; in particular, the double-crankshaft chain along the *c* axis is stretched out considerably over that in the feldspar structure. It should be emphasized that there are two 4.8.8 two-dimensional nets in the H3 framework; one is seen in plan view in Fig. 2B, and the other in side view (dotted lines). The 6.6.6 net is also shown in side view (dashed lines).

The complex bonding properties of the Al and P atoms in the H3 structure, and in particular the existence of alternating 6.6.6 and 4.8.8 two-dimensional nets, suggests that the principle of parsimony should not be taken too strictly in attempts to invent new nets and crystalline materials of potential use in molecular sieve technology. Furthermore, the geometrical flexibility of the two-dimensional component nets of three-dimensional nets (for example, 6^3 net; ref. 20) must be considered in attempts to invent new types of linkages in three-dimensional nets.

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Meteoritic evidence that graphite is rare in the interstellar medium

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This letter discusses the carbonaceous material from several distinct astrophysical environments that has been identified in primitive meteorites. Most of this material is not well-crystallized graphite, but ranges from kerogen-like macromolecular organic matter to poorly graphitized carbon. Of the small fraction of the carbonaceous material which is graphite, most of this may have resulted from the graphitization of macromolecular precursors in the solar nebula. Since graphite is more stable than those precursors, pre-existing interstellar graphite should have survived in meteorites to at least the same extent as did other forms of carbon. I conclude from this dearth of graphite that graphitic carbon was not a major component of the interstellar dust at the time of the formation of the Solar System.

Several forms of carbon are present in the primitive meteorites¹. Some of this carbon may have been produced in the primitive solar nebula¹, some by ion-molecule reactions in interstellar space^{2,3} and a small fraction could have been formed in the expanding shells of red giants⁴, novae⁵ and supernovae⁶. The bulk of meteoritic carbon is 'ordinary' in the sense that it is neither the carrier of an isotopically distinct rare gas component nor itself distinguishable by an 'anomalous' $^{13}\text{C}/^{12}\text{C}$ ratio or associated anomalous $^{15}\text{N}/^{14}\text{N}$ or D/H ratios. The 'exotic' component of primitive meteorites is important not only because of its potential to record subtle clues about the nucleosynthetic process⁷, but also as evidence that some interstellar materials survived the formation of the Solar System and were incorporated relatively intact into meteorites, a possibility suggested by Cameron⁸ as early as 1973.

Anomalous material is that in which the isotopic ratio of an element differs from the standard 'cosmic' isotopic ratio. Both refractory and volatile elements have been shown to be isotopi-

cally anomalous (reviewed in ref. 7). Anomalies in volatile elements are most relevant in the present context because their preservation indicates that some delicate features of pre-solar interstellar grains can still be found in primitive meteorites. Numerous isotopically anomalous rare gas components are known to exist in the meteorites and several of these are thought to pre-date the collapse of the solar nebula. A ^{22}Ne -rich gas component is released from C1 and C2 chondrites at temperatures of 1,075–1,375 K (ref. 9). Recent work¹⁰ has shown that pure ^{22}Ne can be released from a carbonaceous carrier phase at temperatures as low as 875 K. It is now thought that this neon results from the decay of ^{22}Na which condensed on grains formed in the ejecta of a nova. A mixture of xenon isotopes, designated xenon HL, is released from carbonaceous chondrites at temperatures of 875–1,375 K (ref. 11). Xenon HL is enriched in the xenon isotopes 124, 126, 134 and 136 and is thought to have formed in the outflow around a supernova. Another mixture of xenon isotopes, released at 1,375–1,875 K, is enriched in the xenon isotopes 128, 130 and 132 (ref. 4). The composition of this component matches the predicted ratio of xenon isotopes produced via the s-process in red giant stars¹².

It is impossible to overstate the potential influence of secondary metamorphic processes which may have altered the structure or composition of interstellar grains that had survived the 'nebular' phase of Solar System formation. These processes are undoubtedly the source of the abundant graphite found in iron meteorites¹³ and it has been suggested that similar processes may be the source of all meteoritic graphite, even that found in chondrites¹⁴. The existence of the anomalies discussed above, however, indicates that it is possible for some pre-solar grains to have survived relatively intact to the present time. The observed release temperatures indicate that many surviving pre-solar materials were never exposed to temperatures above 1,000 K. Such conditions should not be unfavourable enough to destroy pre-existing interstellar graphite grains and, as noted previously, it has been suggested that the graphite observed in chondrites may have formed in this environment¹⁴.

Most accepted models of interstellar grains consist of various mixtures of graphite, 'silicate' and additional minor components^{15,16}. Graphite is included in these models not only because it can explain the observed peak in the interstellar extinction curve at 220 nm, given certain specific conditions, but also because it is the most stable form of solid carbon in interstellar conditions. (It has the additional advantage that a well-determined set of optical constants is available from the literature.) Millar and Duley¹⁷ have suggested that the 220-nm 'bump' is produced by crystalline magnesium oxide grains, while Hoyle and co-workers¹⁸ have suggested that the observed extinction feature might be caused by various organic materials found in biological systems. More recently, Sakata *et al.*¹⁹ have shown that a 'quenched carbonaceous composite' (QCC), which is a solid soot-like residue produced in a methane plasma discharge, might yield an acceptable fit to the 220-nm extinction peak. Finally, ultraviolet observations by Hecht *et al.*²⁰ have shown that the extinction properties of the dust produced around R Corona Borealis stars are consistent with the properties of amorphous or glassy carbon²¹, rather than with those of graphite. This is especially significant as R Corona Borealis stars are both carbon-rich and hydrogen-deficient ($\text{C}/\text{H} > 100$ in some cases). In such conditions, hydrocarbon formation should be greatly suppressed and there would be relatively few kinetic inhibitions to the formation of graphite²². Failure to form graphite particles in such favourable conditions seems to lessen the probability of their formation in the more common, hydrogen-rich stars that are the source of most interstellar grains²³.

True 'interstellar' carbon has been detected in meteorites² and is distinguished by a greatly enhanced D/H ratio, thought to have been produced through ion-molecule reactions in very cold interstellar clouds²⁴. Such material is not graphite, but consists of a macromolecular kerogen-like polymer¹, a typical composition for which is 76.5% C, 4.5% H, 2.4% N, 4.3% S and 12.3% O (by difference) on a dry, ash-free basis²⁵. Rietmeijer

and Mackinnon¹⁴ have suggested such macromolecular carbon as a possible source material that was metamorphosed to produce the graphite observed in the chondrites.

The facts may be summarized as follows. Graphite is more stable than either the poorly graphitized carbon or macromolecular, kerogen-like organic material observed in meteorites, and therefore should have survived to at least the same degree as did these other forms of carbon. Macromolecular interstellar carbon is found in carbonaceous chondrites, as are the somewhat more fragile 'grains' which contain trapped noble gas components indicative of their formation in various circumstellar environments. Graphite is not observed in the primitive meteorites except in trace amounts quite easily accounted for by the metamorphism of less stable carbonaceous precursors. Such metamorphic processes should not have destroyed pre-existing graphite grains. It therefore seems logical to conclude that graphitic carbon was not a major component of interstellar dust at the time the Solar System formed. Since graphite is not observed to form in R Corona Borealis stars, a circumstellar environment which should be more favourable for the production of graphite grains than a typical carbon-rich circumstellar outflow, we can also infer that newly formed carbon particles are not graphitic. For this reason we might be justified in concluding that graphite is likely to be only a minor component of the modern interstellar dust population. This conclusion may be consistent with a recent suggestion by Hecht²⁶ that the 220-nm bump arises from a population of extremely small graphite grains which would constitute only a small fraction of the total mass of the solid carbonaceous component of interstellar dust.

Most of the questions following from these rather straightforward interpretations can only be answered by detailed laboratory measurements of the appropriate materials and processes. For example, the optical properties of the macromolecular organic component of the meteorites and of QCCs need to be measured as a function of 'metamorphic grade' in order to determine the strength, width and stability of any potentially observable spectral features. Similarly, the effects of the space environment on such materials should be investigated; for example, the effects of irradiation by cosmic rays, X rays or ultraviolet light, the degree of reprocessing induced by shocks and the degree to which thermal annealing can cause 'graphitization'. In addition, it may be possible to use meteoritic data, such as measurements of the abundances of specific 'stellar' isotopic signatures, to place an upper limit on the efficiency with which grains are destroyed in the interstellar medium. Clearly the science of meteoritics can offer considerable data to astrophysicists. Closer cooperation and more communication between these communities is needed to ensure that the available data are used to their fullest potential.

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Formation of mesosiderites by low-velocity impacts as a natural consequence of planet formation

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Mesosiderites are an enigmatic class of stony-iron meteorites composed of roughly equal amounts of pyroxenitic and basaltic silicates and metallic iron-nickel; olivine is rare. We suggest here that, during the period of planet formation, mesosiderites originated by the low-velocity collisions of large metallic core fragments with the surface of a differentiated asteroid-size body. Relative velocities of the asteroids native to this region were low ($\leq 1 \text{ km s}^{-1}$) because it was distant from massive protoplanets. However, a few differentiated asteroids were destroyed by high-velocity collisions with interlopers perturbed by protoplanets into highly eccentric orbits. These collisions reduced mantles and crusts to small silicate fragments but left cores in the form of large, durable metal fragments. Mesosiderite-like pyroxenite-basalt-metal mixtures were formed when large core fragments accreted at low velocities to the regolith of an intact asteroid; the metal contents of other regolithic regions remained low. Most of the olivine in mesosiderite and howardite breccias we attribute to the mantles of the disrupted parent bodies. The low ($12\text{--}20 \text{ mg g}^{-1}$) olivine contents indicate that the amount of debris accreted from disrupted asteroids was small relative to the volumes of the regoliths.

Because differentiated bodies with basalt-rich crusts are expected to have ultramafic mantles and metal-rich cores, models in which mesosiderite basalt and metal are proposed to have originated in the same body cannot easily explain the scarcity of olivine in these stony-iron meteorites. For example, one recent suggestion is that basaltic crustal blocks foundered and sank through a molten peridotite mantle and were then invaded by molten metal from the core¹. This model requires an implausible thermal history to explain the survival of basalt (melting temperature $\sim 1,400 \text{ K}$) exposed to molten mantle and core material ($T \geq 1,800 \text{ K}$).

The least recrystallized mesosiderites (Fig. 1a) are impact breccias, consisting of poorly sorted, angular, igneous silicate clasts, recrystallized breccia clasts and metal nodules in a matrix of metallic Fe-Ni and silicate grains²⁻⁵. The mesosiderites that have undergone the greatest thermal processing are clast-laden impact melt-rocks in which immiscible silicate and metal-sulphide liquids coexisted^{5,6}. Evidence for brief high-temperature events, inferred from mineralogical disequilibrium⁷, also support the proposal that mesosiderites are impact products.

The model invoking core accretion to basalt-rich regolith⁸ has not gained acceptance because relative impact velocities in the asteroid belt ($\sim 5 \text{ km s}^{-1}$) are too high to allow a projectile/target mass ratio near unity. Although previous workers⁹ recognized that low ratios would be expected at lower relative velocities ($\leq 1 \text{ km s}^{-1}$), no scenario leading to such low velocities has been proposed.

The differentiation of planetary bodies normally results in the extrusion of basaltic lavas. Pyroxenitic cumulates could lie immediately below these basalts. Spectral evidence of basalt on the asteroid 4 Vesta^{10,11} suggests that many asteroid-size bodies that formed within Vesta's orbit (2.4 AU from the Sun) were differentiated because most plausible asteroidal heat sources

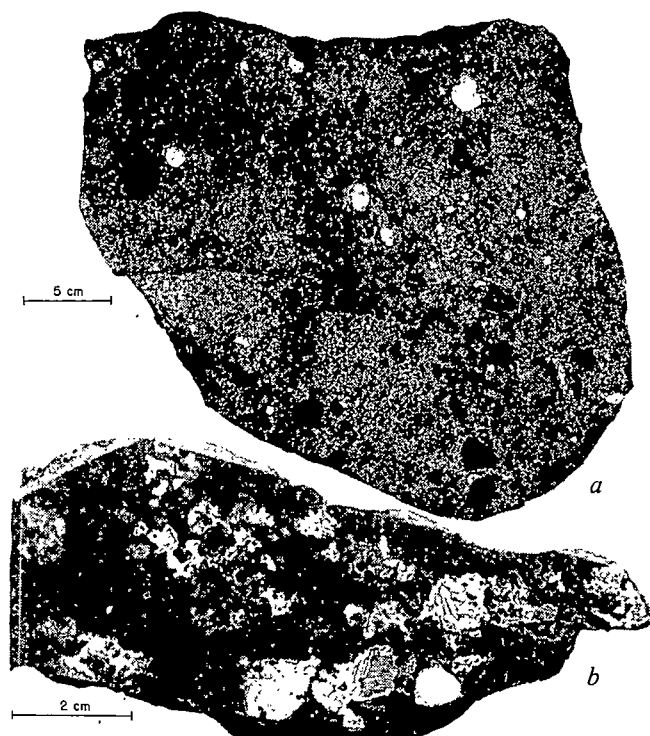


Fig. 1 a, Photograph of the Mt Padbury subgroup 1A mesosiderite (Smithsonian Institution specimen USNM 3454) showing angular silicate clasts, metal-rich breccia clasts and metal-sulphide nodules within a metal-silicate matrix. Metal grains have been finely comminuted and intimately mixed with silicates through a series of impacts. b, Photograph of the Estherville subgroup 3/4 A mesosiderite (Smithsonian Institution specimen USNM 1025) showing many large rounded metal nodules within a silicate matrix. Estherville suffered less extensive impact reworking than many other mesosiderites.

increase in efficiency with increasing proximity to the Sun. This is obvious for Sun-centred sources such as solar wind-induced electrical currents, but is also true for impact heating, because relative velocities are larger nearer the Sun. Heating of planetesimals by ^{26}Al is more efficient for objects forming earlier in the history of the Solar System; a simple model proposed by Safronov¹² suggests that planetesimals formed earlier nearer the Sun. Internal isochrons determined for eucrites and howardites¹³⁻¹⁶ show that the melting of these bodies occurred within 50 Myr of the formation of chondrites.

The oxygen-isotope compositions of the possibly martian SNC meteorites are only 0.4% $\delta^{17}\text{O}$ above the terrestrial fractionation line¹⁷, supporting the suggestion that primitive bodies that formed in the inner Solar System had O-isotope compositions near this line¹⁸. The O-isotope compositions of mesosiderites and related differentiated meteorites (eucrites, howardites and diogenites) lie equally near the terrestrial fractionation line¹⁹, suggesting that these meteorites also formed in the inner Solar System, perhaps inside 1.5 AU.

Wasson and Warren²⁰ cited evidence in support of the idea that the Moon formed by the circumterrestrial accretion of silicate fragments from differentiated asteroid-size bodies. It seems plausible that, at that time, more than 90% of the bodies in heliocentric orbits having radii near 1 AU were differentiated. We suggest that a similar fraction was differentiated at the mesosiderite formation location. Eucrites, howardites and mesosiderites are unlikely to be from Vesta²¹, because this asteroid is only one of a very large set of differentiated bodies. Fragmented members of this set are far more plausible parents.

According to the scenarios for planet formation proposed by Safronov¹² and Wetherill²², about 100 Myr was required between the stage of planetesimal formation by gravitational collapse to the growth of the inner planets to 95% of their present sizes.

At some epoch during planet formation, most bodies were in low-inclination, nearly circular orbits and average relative velocities in the inner Solar System were $\sim 1 \text{ km s}^{-1}$. Such low velocities would be preserved until 10^{26} -g, Mars-size bodies in eccentric orbits passed through the region. If some distant proto-planets were already Mars-size, some asteroid-size bodies in their vicinities could have been perturbed into eccentric orbits that caused them to pass through the mesosiderite formation region with relative velocities of $5\text{--}10 \text{ km s}^{-1}$. Impacts with these high-velocity interlopers fragmented a few differentiated native bodies into crust, mantle and core debris, the relative masses of these materials probably being about 10%, 65% and 25%, respectively. In those cases in which the high-velocity interloper was much smaller than the body it destroyed, the fragments of both bodies ended up in heliocentric orbits that were nearly circular. Weak crusts and mantles broke into small pieces; the more durable metal cores remained largely intact.

The accretion of a metallic projectile onto a basalt-rich regolith at a velocity of $\sim 1 \text{ km s}^{-1}$ should form large amounts of materials in which the metal/silicate ratio is approximately unity. Some blocks of metal or silicate would not be reduced to the millimetre size typical of mesosiderites, but those with dimensions $\geq 20 \text{ cm}$ might not be recognized as mesosiderites if they fell to Earth as individual meteorites. The textures of some mesosiderites attest to several generations of breccia formation (Fig. 1a); thus, a series of impacts may have reduced the mean grain sizes to those observed. Estherville (Fig. 1b) contains large (1–3 cm diameter) metal nodules and has probably experienced less extensive impact reworking than mesosiderites possessing fine-grained intimate mixtures of metal and silicate (for example, Mt Padbury, Emery, Mincy). Continuing impacts may have generated sufficient heat to cause metamorphism or even incipient melting in some mesosiderites.

Because, in a typical asteroid, the mass of the peridotitic mantle is expected to be about three times greater than the mass of metal in the core, we expect the proportion of accreted olivine to be more abundant than that of metal in the regolith. If olivine was mainly accreted as small debris, the amount of olivine in mesosiderites can be used as a measure of the fraction of the regolith composed of material derived from the accretion of one or more disrupted differentiated bodies. The median olivine content of mesosiderite silicates ($\sim 16 \text{ mg g}^{-1}$)²³ implies that $\sim 25 \text{ mg g}^{-1}$ of the regolith accreted from this source. The howardite regolith breccias provide a test of this scenario; they have chemical and O-isotope compositions nearly indistinguishable from those of the silicate portions of the mesosiderites, and probably formed either on the mesosiderite body or on a neighbouring body. Their median olivine content ($\sim 12 \text{ mg g}^{-1}$; ref. 24) is similar to that of mesosiderites.

Large ($\sim 1 \text{ cm}$) olivine clasts in Mt Padbury, Mincy, Pinnaroo, Vaca Muerta, and West Point have compositions ranging from Fa_{8-12} (refs 2, 25–27); in Emery such clasts range from Fa_{18-28} (ref. 26). The compositional range of the large clasts is narrower than that of all mesosiderite olivine clasts (Fa_{9-42})²⁸ and is similar to that (Fa_{10-30}) calculated for the lunar mantle from magma ocean models²⁹. These observations are consistent with a mantle origin for mesosiderite olivine. Olivine composition is best preserved in large clasts; fine-grained olivine tended to become more ferroan as a result of equilibration with the much more abundant indigenous mafic silicates in the regolith.

If a negligible ($<10\%$) fraction of mesosiderite olivine is indigenous, the observed 16 mg g^{-1} of mantle olivine implies that $\sim 5 \text{ mg g}^{-1}$ of the regolith consisted of core materials. This low abundance is not inconsistent with the fact that metal accounts for $\sim 500 \text{ mg g}^{-1}$ of mesosiderites if we assume that the metal from disrupted asteroids primarily resided in large ($>100 \text{ m}$) fragments. Mesosiderites formed where these objects accreted, whereas metal contents of other regolithic regions remained low, as in howardites. Because the metal content of the mesosiderites is 100 times greater than that estimated for the entire regolith, mesosideritic regolith is inferred to have formed only 1% of the parent body surface.

There are two compositional subgroups (A and B) of mesosiderites, possessing different modal abundances of orthopyroxene, plagioclase and tridymite^{5,23,30}. The somewhat different mean Ir/Ni ratios ($\times 10^5$) of the metal in these groups (3.6 ± 0.91 and 5.1 ± 1.2 , respectively)⁸ suggest that metal core fragments accreted to at least two distinct regolithic regions.

Mesosiderite metal compositions are roughly chondritic^{8,31}. Most cores seem to have formed by fractional crystallization³², but most core fragments are approximately chondritic. The Rayleigh equation can be used to show that nearly half the volume of a metal core would have siderophile ratios within a factor of 3 of the chondritic ratio. This is the case even for elements having extreme solid-liquid distribution coefficients (k) such as Au ($k=0.4$) and Ir ($k=3$).

The moderately high abundance for mesosiderites among meteorite falls can be accounted for by biases resulting from parent body ejection, space erosion and the rigors of atmospheric passage, all of which favour the tough, metal-rich mesosiderites over the more friable howardites.

The only mesosiderite property seemingly incompatible with a near-surface origin is the apparently slow cooling rate below $\sim 800 \text{ K}$ inferred from metallographic evidence. Powell³ and Hewins³³ estimated this rate to be $\leq 1 \text{ K Myr}^{-1}$. Nevertheless, it is possible that it was not monotonic cooling, but low-temperature ($600\text{--}700 \text{ K}$) annealing or thermal cycling for long time periods that produced the metallographic structures. Even if slow cooling actually occurred, the burial depth^{34,35} could be as low as $\sim 15 \text{ km}$ if the thermal diffusivity was similar to that of the lunar regolith ($10^{-4} \text{ cm}^2 \text{ s}^{-1}$)³⁶. Such a regolith depth is plausible for a body accreting material at low relative velocities.

Thus, it seems probable that mesosiderites formed when asteroid-size bodies were still growing. We suggest that many such bodies in the inner Solar System differentiated before accumulation was complete. If a few of these differentiated bodies were disrupted by high-velocity interlopers, it is inevitable that some of their fragments would accrete at low velocities to the basalt-rich regoliths of bodies that avoided disruption. Olivine contents reflect the fraction of the regolith from this source. Mesosiderite-like materials were produced in those rare regolith locations where large core fragments accumulated. Selection biases enhanced the flux of mesosiderite-like breccias relative to that of howardite-like breccias onto the Earth's surface.

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A large-scale meridional circulation in the convective zone

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Many attempts have been made to detect, at the solar photospheric level, large-scale motions related to the Sun's global convection and dynamo (see ref. 1). Because these motions are likely to be influenced by solar rotation, one might expect that they would take the form of 'bananas' aligned with the solar rotation axis², as a result of the Taylor-Proudman constraint. The Paris observatory has one of the longest series of spectroheliograms, dating back to 1919. In view of the current interest in the history of solar activity, we have started to digitize the collection³. This allows us to measure rotational rate and meridional drift for individual sunspots with an accuracy of a few ms^{-1} for the best seeing conditions at Meudon. A new phenomenon, which may lead us drastically to revise our ideas on the large-scale convection of the Sun, can immediately be seen. Newly-born sunspots trace a roughly axisymmetric meridional circulation, in the form of four zonal bands, with relatively large velocity amplitude. If these drifts are assumed to trace fluid motions at some level in the solar envelope, the resulting circulation pattern cannot be associated with the 'banana' cells mentioned above. We report here results based on two sets of data; one involves a detailed analysis of two periods, each covering five solar rotations while the other is essentially qualitative and results from eye-estimates of the spot meridional drifts over much longer time sequences.

The first period (Carrington rotations 1547 to 1551) spans sunspots at the maximum of the solar cycle 20th, the second (Carrington rotations 1587 to 1591) corresponds to the declining phase. Twenty new bipolar groups were born on the visible disk for each period. A third sequence covering the summer of 1967 (ascending phase) has been partially analysed and confirms the following results.

A meridional circulation pattern can be seen in Fig. 1. This is due to young sunspots, and it is larger than in any possible experimental error. It consists of four latitude bands, two in each hemisphere, each pair with opposite directions of circulation. Furthermore, the sense of the meridional drift within each band reverses during the solar cycle. For example, in 1969, sunspots born between latitudes 10° and -21° move towards the equator while sunspots born beyond these latitudes move towards the poles. In 1972, sunspots born within $14^\circ 5'$ and -17° move towards the poles while outside this range, they move towards the equator. Meridional velocities range from zero to 100 m s^{-1} (1° day^{-1} corresponds to 130 m s^{-1}). This value is compatible with convective velocities predicted for giant cells⁴.

One property of the meridional circulation is that it is roughly axisymmetric. Although sunspots are often found at certain preferred longitudes, our data sample a fairly wide range of longitude, especially in the declining phase of the cycle; the observed drifts do not seem to depend on longitude. However, this property of axisymmetry should be confirmed over a larger statistical sample.

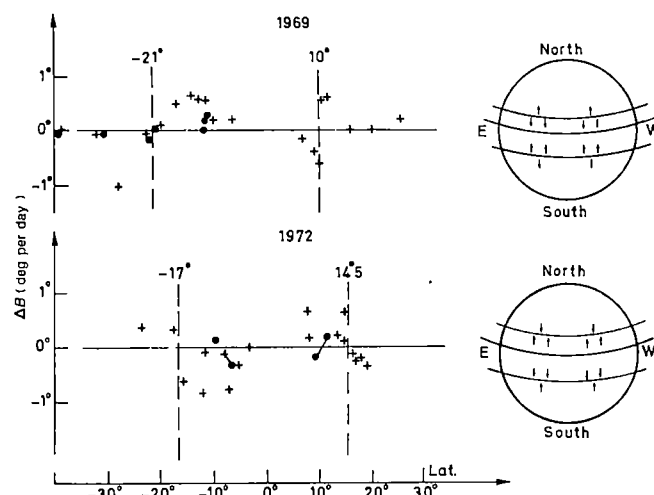


Fig. 1 The meridional circulation traced by young sunspots plotted as a function of latitude for the 1969 and 1972 sequences. \times , Leading sunspots; \bullet , following sunspots. In the northern hemisphere, a positive meridional drift ($\Delta B > 0$) indicates that spots are moving towards the north pole, while a negative drift ($\Delta B < 0$) corresponds to a displacement of spots towards the equator.

The rotational rate of young spots is nearly constant with latitude. The synodic leader's rate ($14^\circ 22'$ per day in 1969) is larger than the follower's, as expected from the rise of the magnetic loop (the difference is about $1^\circ 5'$ for the first day and drops rapidly during the next days). The mean deviation, σ , of $0^\circ 7'$ for the leaders and $0^\circ 5'$ for the followers is mainly due to some low latitude spots (at the boundary of the two latitude bands) and does not reflect the differential rotation. Note that the large σ value is partly due to the difficulty of observing young spots on the first day of their emergence. The rotation rate, Ω , at the depth where the spots are anchored should be determined from the centre of gravity of the emerging magnetic loop. But because this is unknown, we used the midpoint between the leaders and the followers. Ω is then approximately equal to the surface equatorial rate. This result is consistent with the deep solid rotation implied by the observed rotational splitting of global oscillations^{5,6}. It also indicates that young spots are better tracers of the deep convective layers than older spots which exhibit a pronounced differential rotation^{7,8}.

The average rotational rate increases by 5% between 1969 and 1972, an increase comparable with the σ value of young spot rotation. This effect is in qualitative agreement with the report⁹ that the solar rotation is faster at the minimum of the cycle. Our sample is too small to allow a quantitative comparison to be made, however.

The question then arises, of whether this meridional circulation of the young spots traces fluid motions at some level in the solar envelope. There are several arguments which suggest that it does.

First, sunspots usually emerge at the surface as simple dipolar magnetic features. When the magnetic loop rises, the leading and the following spots diverge and, a few days later, the following spot starts to fragment and gradually disperses into the general surrounding magnetic field, leaving only the leading spot as an identifiable feature. If one accepts that sunspots are anchored deeply in the convective zone¹⁰, then the drag exerted by the surrounding plasma on the magnetic tubes should be much stronger when the magnetic structure is more complex, that is in the later stage of the spot's life. Thus, newly-born spots are probably the best tracers of fluid motions in the deep convection zone where the dynamo process is supposedly acting.

Second, the spot circulation seems to be related to the large-scale, long-lived, magnetic structures outlined by the $H\alpha$ filaments. The procedure used to look at these structures is

Fig. 2 Evidence of azimuthal rolls (dashed arrows) from the observed meridional circulation of young sunspots (solid arrows) and the drift of long-lived $H\alpha$ filament trajectories (solid lines). The direction of rotation seems to be associated with the magnetic polarity. A new roll appears every 2 or 3 yr. Sunspot activity seems to be concentrated near the updrafts (u) more than near the downdrafts (d).

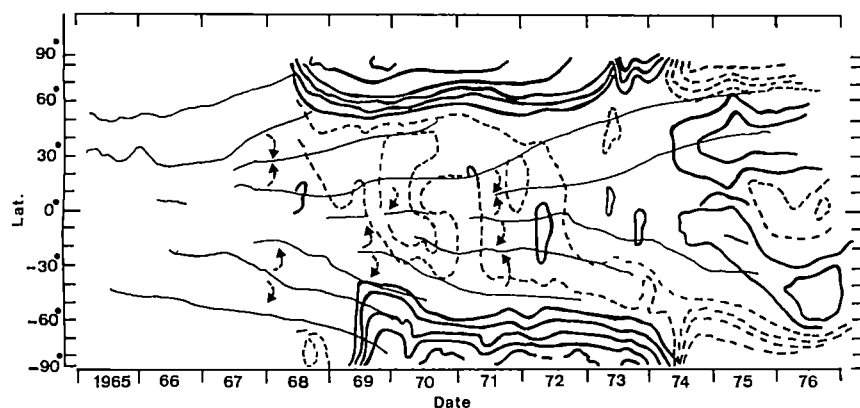
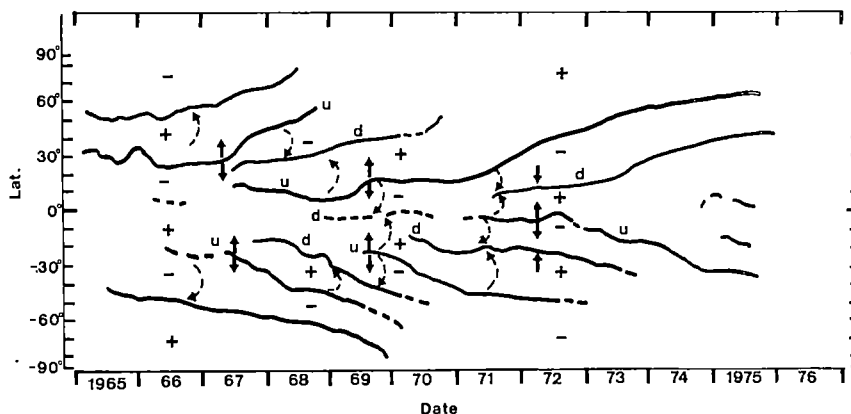


Fig. 3 Contour maps of differences from average differential rotation (intervals are ± 32 , ± 16 , ± 8 and $\pm 4 \text{ m s}^{-1}$) obtained by Snodgrass and Howard¹². Dashed lines and solid lines correspond to smaller and larger rotational shear. The azimuthal rolls (thin lines) seem to influence the angular momentum transport by reducing the rotational shear. The direction of rotation of the azimuthal rolls is indicated by the dashed arrows.

similar to that used by Makarov¹¹. A mean latitude is assigned to every filament band present on the solar surface during one rotation. A three-rotation-average for each band is then taken. The filaments bands drift with time. If we accept MacIntosh's claim that $H\alpha$ filaments outline the boundaries of regions of unipolar magnetic fields, we can determine an azimuthally-averaged magnetic pattern of alternating polarity. We have disregarded short-lived $H\alpha$ filaments which introduce noise in the data (in contrast with Makarov). As shown in Fig. 2 (see also ref. 11), the magnetic pattern is not random and consists of large-scale azimuthal magnetic regions. Moreover, the magnetic pattern drawn by $H\alpha$ filaments (solid lines in Fig. 2) fits the critical latitudes of the zonal meridional circulation. It is difficult to see how such a pattern could be established other than by large-scale motions. The remarkable coincidence between the circulation pattern and the magnetic pattern suggests that we are looking at azimuthal rolls.

Third, the circulation pattern reflected by young spots interacts with the transport of angular momentum. Snodgrass and Howard¹² have noticed periodic changes in the differential rotation close to sunspot maximum. By superimposing the azimuthal pattern on their data (Fig. 3), it can be seen that the presence of rolls is restricted to the latitudes which have less rotational shear. This is a natural consequence of a meridional circulation in the form of several counter rotating cells.

From these converging arguments, a new picture of the dynamics of the convective zone and the dynamo action emerges. Four to six azimuthal rolls are present, having widths of $\sim 20^\circ$ – 40° with a lifetime of several years. The direction of rotation of a roll is associated with its magnetic polarity. Every 2 or 3 yr, a roll dislocates and a new one appears. The appearance of rolls and their dislocations seems to be related to sunspot activity. In particular, new spots are found mostly in the vicinity of the boundaries between two adjacent rolls. They arise preferentially where the two rolls are diverging rather than converging. This suggests that magnetic fields are expelled by upward motions¹³. After each bifurcation of a roll (in 1967, 1969, 1970 and 1971), a new one appears and the updraft situation moves closer to

the equator. This could explain the shift in latitude observed in the so-called Maunder butterfly diagram of sunspots.

Is there any connection with the giant convective cells which have been invoked³ but never detected? It is hard to give a definitive answer as our method of analysis is biased towards larger-scale motions and precludes the detection of smaller-scale motions such as the giant cells mentioned previously. However, the velocities we have observed are comparable in magnitude to convective velocities calculated for the deep convection zone⁴. This is a very strong argument in favour of a convective origin for the meridional circulation of young spots. If this is the case, we must relax the Proudman–Taylor's constraint. The radial rotational shear, inferred from helioseismology⁶, is too weak in the convective zone to be relevant. On the other hand, a toroidal magnetic field of 10^3 G could force the large-scale convection to take the form of rolls aligned with the azimuthal direction¹⁴.

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Parity-violating energy differences of chiral minerals and the origin of biomolecular homochirality

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The biochemistry of terrestrial organisms is based on chiral (handed) molecules, with one of the two possible series of enantiomers (mirror-image isomers) being predominant. Specifically, terrestrial biochemistry is homochirally supported by L- α -amino acids and D-sugars to the almost complete exclusion of the enantiomeric D- α -amino acids and L-sugars. This particular homochiral selection may be a result of very small differences between the electronic energies of enantiomeric prebiotic molecules due to the parity-violating weak interactions. The energy differences are, however, so small for simple chiral molecules, that the propagation of homochirality would require a dissymmetry amplification mechanism involving both large quantities of reactants and a long reaction time. An alternative theory, presented here, considering the effects of the parity-violating weak interactions in crystalline enantio-selective prebiotic catalysts, such as the clay silicates, may require considerably less amplification.

The natural competitive selection of a homochiral biochemistry is accounted for generally by elaborations of the stereochemical 'lock-and-key' hypothesis of Fischer¹, but conventionally the terrestrial selection of the L- α -amino acid and D-sugar series over the classically equally viable enantiomeric alternative has been ascribed to chance. The efficient formation of prototype biomolecular polymers during the prebiotic-biotic transition requires the existence of a chemistry approaching homochirality²⁻⁴, and, until recently, the mechanisms proposed for its evolution from a prebiotic racemic geochemistry were equivocal, either of the two enantiomeric homochiral chemistries being selected with equal probability⁵. The violation of parity by the weak interactions provides an alternative, globally consistent, basis for the systematic and determinate selection of the observed homochirality without recourse to chance events.

Through the connection established with electromagnetism^{6,7} the parity-violating weak interactions cause a chiral molecular system and its enantiomer to have slightly inequivalent energies⁸⁻¹¹. Effectively there is a small parity-violating energy difference (PVED), ΔE_{pv} , between the corresponding state energies of a chiral system and its enantiomeric system, which preferentially stabilizes one with respect to the other. The PVED may occur for any chiral system, including transient forms, and can, therefore, manifest itself as an imbalance in the quantities of enantiomeric species in classically racemic equilibrium or as an inequivalence in the rate constants of enantiomeric reactions.

The intrinsic PVED between enantiomeric species and reactions involved in the prebiotic-biotic transition may have specifically selected the known terrestrial homochirality. Recent calculations indicate that the natural L-enantiomers of a series of α -amino acids are stabilized relative to their corresponding unnatural D-enantiomers for the conformations preferred in aqueous media¹²⁻¹⁵. A similar stabilization of the natural enantiomers has been determined for a set of polypeptide/protein fragments in both the α -helix and β -sheet conformations^{12,13} and also for a possible prebiotic reaction to form a precursor of L-alanine¹⁶.

The calculated values of PVED for small chiral molecules are exceedingly small, typically $\sim 10^{-20}$ atomic units (a.u., equivalent to 'hartrees'), although other conceivable systematic chiral influences are generally even weaker. Nonetheless, several kinetic mechanisms that amplify dissymmetry to yield homochirality have been suggested¹⁷⁻²⁰, and it has been determined that even a PVED as small as $\sim 10^{-20}$ a.u. may be sufficient as a selector. However, latest estimates²⁰ of the reaction conditions

required for such an amplification are a lake of volume 4×10^9 litres ($1 \text{ km} \times 1 \text{ km} \times 4 \text{ m}$) which remains homogeneous for some 10,000 yr. While such conditions are possible in the prebiotic era, they must be regarded as exceptional.

The essential difficulty is the minuteness of the intrinsic PVED for small prebiotic chiral molecules. Giant chiral molecules such as polymers, and especially crystal lattices, may have much larger PVED, and may exhibit the resulting dissymmetries at a detectable level without the intervention of amplification mechanisms. For example, the ratio of the numbers of left (*l*) and right (*d*)-handed enantiomorphous crystals (*l/d*), each with *n* unit cells, under chemical equilibrium at temperature *T* is given by

$$l/d = \exp(-\Delta E_{pv}^{(n)}/kT) \quad (1)$$

where *k* is the Boltzmann constant, and the PVED, $\Delta E_{pv}^{(n)}$, is the energy of a (*l*)-enantiomer crystal relative to a (*d*)-enantiomer crystal of the same size (*n*). Taking the first-order approximation^{21,22} that the overall PVED, $\Delta E_{pv}^{(n)}$, has a linear relationship with the number of unit cells, *n*, it can be expressed in terms of the mean PVED per unit, ΔE_{pv}^I , or the advantage factor $\epsilon = -\Delta E_{pv}^I/kT$

$$\Delta E_{pv}^{(n)} = n\Delta E_{pv}^I = -nekT \quad (2)$$

whereupon the dissymmetry ratio becomes

$$l/d = e^{n\epsilon} \approx 1 + n\epsilon \quad |n\epsilon| \ll 1 \quad (3)$$

A corresponding result can be obtained from a kinetic approach using the progressive accumulation model²¹, the PVED then referring to the transition states for crystal growth. The enantiomeric excess, Ω_{crys} , a more convenient measure of the dissymmetry, is related by

$$\begin{aligned} \Omega_{crys} &= \frac{l-d}{l+d} \\ &= \frac{e^{n\epsilon} - 1}{e^{n\epsilon} + 1} \\ &\approx \frac{n\epsilon}{2} \quad |n\epsilon| \ll 1 \end{aligned} \quad (4)$$

For the large number of units, *n*, possible in crystal lattices, even very small non-zero PVED per unit can be enhanced to give a detectable difference in the quantities of left- and right-handed forms of a chiral crystal. Remarkably, such a difference has been documented for naturally occurring quartz²³ where, over a total collection of 16,807 crystals, a 1% enantiomeric excess of *l*-(-)-quartz has been found at all the locations sampled in the Americas, Europe and Asia, with a 90% statistical confidence level that the bias was not a matter of chance. Using a value of the advantage factor $\epsilon \approx 10^{-17}$, appropriate for a unit PVED, ΔE_{pv}^I , of a small chiral structure composed of light atoms ($\sim 10^{-20}$ a.u.), the 1% enantiomeric excess of terrestrial quartz requires $n \approx 10^{15}$ in equation (4). A quartz crystal containing 10^{15} unit cells has the dimensions $\sim 0.1 \text{ mm}$ along each main edge, and is, therefore, very easily attainable. Consequently, the observed natural quartz bias may be evidence of the existence of PVED effects in the physical world, although, at present, it is impractical to perform the necessary calculations of PVED for quartz to confirm this.

An alternative explanation, that the excess of *l*-(-)-quartz was laid down in the post-biotic period and was influenced by the already existing biomolecular homochirality is unlikely. The elevated temperatures associated with the hydrothermal crystallization of quartz are unfavourable, the α -amino acids, for example, racemizing at $\sim 160^\circ\text{C}$ in aqueous solution with a half-life of only a few hours²⁴. Some experimental evidence has also been reported²⁵ on the transference of β -ray dissymmetry to the crystallization of the chiral salt sodium ammonium tartrate

under radiolysis, although its implications for the crystallization of quartz and other minerals are unknown.

Quartz is only one of the many chiral crystalline silicates to be found as minerals of the Earth's surface, and each may exhibit, to differing degrees, analogous enantiomeric excesses. The role of terrestrial minerals, especially the layer-structured silicate clays, in the prebiotic-biotic transition has long been discussed^{26,27}, and interest has recently been furthered by the discovery that clays can absorb mechanical and electromagnetic energy and release it slowly over periods of days²⁸. The most obvious method by which minerals can influence the prebiotic-biotic transition is through their action as catalysts, however to produce an enantiomeric excess in chiral product molecules from racemic/achiral reactants; it is necessary not only to have an enantiomeric excess in the chiral catalyst, but also that it must act enantio-selectively. This enantioselectivity is common, and is illustrated by the preferential adsorption of L-alanine from a racemic mixture by L-(−)-quartz^{29,30}.

For a simple catalytic reaction transforming racemic/achiral reactants into the L- and D-forms of a chiral product with rate constants k^L and k^D respectively, the degree of enantio-selectivity of a particular catalyst can be represented by its enantio-selectivity factor, S

$$S = \frac{k^L - k^D}{k^L + k^D} \quad (5)$$

The enantio-selectivity factors for the left-handed, S_L , and right-handed, S_D , forms of otherwise equivalent chiral catalysts will be different but related by $S_L = -S_D$.

The combination of an enantiomeric excess, Ω_{crys} , of a chiral catalyst (composed of crystals of equal size and surface area), with an enantio-selectivity $S = S_L$ ensures that for such a reaction there is an overall enantiomeric excess, Ω_{prod} , in the numbers of left(L)- and right(D)-handed product molecules formed from a racemic/achiral reactant mixture which is given by

$$\Omega_{\text{prod}} = \frac{L - D}{L + D} = S\Omega_{\text{crys}} \quad (6)$$

If the catalyst is either racemic ($\Omega_{\text{crys}} = 0$) or not enantio-selective ($S = 0$), there is no transference of any chiral bias.

Taking $\Omega_{\text{crys}} = 0.01$, in keeping with the observed 1% enantiomeric excess of terrestrial quartz, and a conservative 0.1% enantio-selectivity ($S = 10^{-3}$), the resulting enantiomeric excess in the chiral product molecules will amount to some 0.001% ($\Omega_{\text{prod}} = 10^{-5}$); this is considerably larger than the corresponding enantiomeric excess due to the inherent PVED of the product molecules themselves, which typically only amounts to $|\Omega_{\text{prod}}| \approx 10^{-17}$ at ambient temperatures.

Whilst the chemistry resulting from chirally biased catalysis is not totally homochiral ($|\Omega_{\text{prod}}| = 1$) it is a great improvement over alternative models, and would require only minor subsequent dissymmetry amplification, which could be achieved by the various proposed mechanisms¹⁷⁻²⁰ using smaller reaction volumes and less time than that required by comparable models. Alternatively, chirally biased catalysis by minerals could form an integral part of a dissymmetry amplification mechanism, further ensuring the rapid propagation of homochirality. Enantio-selective frameworks in possible dissymmetry amplification processes have been experimentally demonstrated by the chirally biased occlusion of α -amino acids into glycine crystals³¹ and also in template-directed oligomerization of biomolecules².

Throughout the above discussion it has been implicitly assumed that the enantiomeric excess and selectivity of the catalyst are such that the bias is towards the observed natural enantiomers of the principal biomolecules rather than their unnatural enantiomeric counterparts. Unfortunately, at present, the validity of this assumption cannot be determined because there is little detailed knowledge of the chemical processes of the prebiotic-biotic transition, and thus the specific relevant catalysts and reactions cannot be identified. Nonetheless, the model's ability to produce relatively large enantiomeric excesses

with ease warrants careful consideration and further investigation.

In conclusion, the indirect globally systematic influence of the weak interactions on the prebiotic-biotic transition, through the parity-violating energy differences in crystalline enantio-selective catalysts, may have specifically selected the observed terrestrial biomolecular homochirality. The role of clays in the prebiotic-biotic transition is still under debate, but their inherent ability to ensure the emergence of a homochiral biochemistry is further evidence in their favour.

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New higher primates from the early Miocene of Buluk, Kenya

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The important early Miocene site of Buluk, Northern Kenya¹, was re-surveyed in 1983. As a result, the faunal list for this site has been expanded and, as we report here, now includes three hominoid and at least one cercopithecoid species. Some of the species were known previously only from North African sites, but others are typically East African. Only *Proconsul* species have been found before at other East African Miocene sites of equivalent age, but the largest hominoid species from Buluk is an early species of *Sivapithecus*.

The Buluk fossil site (36° 36' E, 4° 16' N) is a small exposure of claystones with coarse sandstone and conglomerate channel fills belonging to the Buluk Member of the Miocene Bakate Formation²⁻⁴. The fossils are found almost exclusively in the channel deposits and are often associated with silicified wood. K/Ar dates on a basalt just above the fossiliferous sediments show that the fossils are older than 17.2 Myr⁴. The 1983 expedition collected new specimens and the faunal list previously given by Harris and Watkins¹ has been expanded and consolidated (see Table 1). Of particular importance is the presence of *Libycochoerus massai* and the crocodile *Tomistoma*, known before only from North Africa. Some fossil fruits were found which

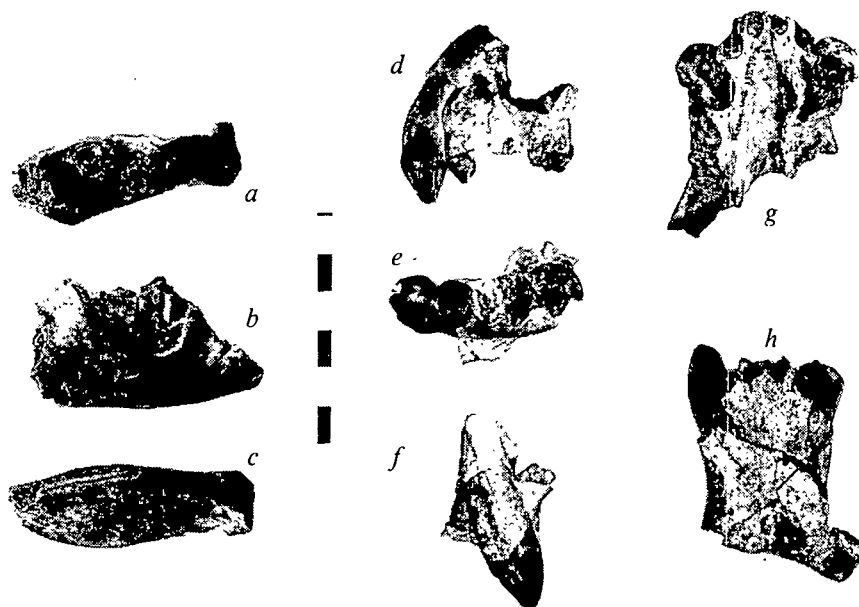


Fig. 1 a-c, Occlusal, right lateral and inferior views, respectively, of KNM-WS 124 (right mandible). d-f, Left lateral, occlusal and anterior views, respectively, of maxilla, KNM-WS 11599. g, h, Occlusal and anterior views, respectively, of mandible, KNM-WS 11599.

are similar to those reported from Rusinga Island as belonging to the form genus *Anonaspermum*⁵.

Three hominoid species are now known from Buluk (see Table 2). A small hominoid about the size of *Micropithecus clarki*⁶ is represented by KNM-WS 12610, a weathered mandible with very worn M_{2-3} . A medium-sized species is known by a right mandibular body with the roots of M_{2-3} and an isolated right M^1 (KNM-WS 12602 and 12608). These remains are about the size of the type specimen of *Kenyapithecus wickeri* from Fort Ternan⁷. On the basis of size there is no doubt that the middle-sized form is a separate species from the better represented large hominoid.

The large hominoid is known by gnathic fragments. The two most complete specimens are described here and illustrated in Fig. 1. KNM-WS 124 is a right mandibular body preserved from the midline at the symphysis to a break behind the M_3 roots.

The crowns of the molars are absent and M_1 was lost in life, as judged from the lack of roots and a large patch of alveolar resorption beneath the distal position of the tooth and beneath M_2 . The base at the symphysis is thickened so that the mental foramen lies near the edge of a hollowed surface under the premolars. The body is deepest under M_2 (38 mm) and is 19 mm thick at this point. The lingual surface is relatively flat, but hollowed in the middle behind M_3 . The buccinator groove is wide (~10 mm) and the take-off of the ramus is twisted so that its surface faces laterally and posteriorly. The external buttress continuing from the anterior edge of the ramus swings in a smooth arc under the molar roots and beneath the mental foramen to continue as the canine ridge. The anterior digastric rugosity is faint. The lengths of M_2 and M_3 have been estimated from their roots at 13 and 15 mm, respectively. This specimen is remarkably similar in parts preserved, size and morphology to GSP 11706 from the Potwar Plateau, Pakistan⁸.

Specimen KNM-WS 11599 is an associated left maxilla and anterior mandible. The mandible fragment has the alveoli for the incisors, roots of the left canine and premolars and the crown of the right canine preserved. The symphysis and the left body to a level under M_1 are present. The symphysis is deep. The alveolar plane angles posteriorly at ~45° and is highly excavated, rising steeply on either side from a midline sulcus. There are clearly defined superior and inferior tori, the latter

Table 1 Faunal list

Pisces	Clariidae <i>Protopterus</i> sp.
Reptilia	Pelomedusidae Trionychidae <i>Crocodylus</i> cf. <i>niloticus</i> <i>Tomistoma</i> sp.
Mammalia	<i>Prohylobates</i> sp. cf. <i>Micropithecus clarki</i> cf. <i>Kenyapithecus wickeri</i> <i>Sivapithecus</i> sp. Indeterminate rodents <i>Hyainalouros nyanzae</i> Very large amphicyonine <i>Gomphotherium</i> cf. <i>angustidens</i> New genus and species of gomphothere <i>Prodeinotherium hobleyi</i> <i>Megalohyrax championi</i> <i>Dicerorhinus leakeyi</i> <i>Chilotheridium pattersoni</i> <i>Aceratherium acutirostratum</i> <i>Hyoboa</i> sp. <i>Diamantohyus africanus</i> <i>Libycochoerus massai</i> <i>Lopholistriodon</i> cf. <i>moruoroti</i> <i>Canthumeryx</i> cf. <i>sirtensis</i> <i>Dorcatherium chappuisi</i> <i>Dorcatherium pigotti</i>

Table 2 New higher primates from Buluk

cf. <i>Micropithecus</i>	
KNM-WS 12717	Mandible with M_{2-3}
KNM-WS 12636	Right femur
cf. <i>Kenyapithecus wickeri</i>	
KNM-WS 12602	Right mandible
KNM-WS 12607	Upper right M_3
<i>Sivapithecus</i> sp.	
KNM-WS 124	Right mandible
KNM-WS 125	Left mandible
KNM-WS 11599	Associated mandible and left maxilla with lower right C, upper C, P3, M_2
KNM-WS 12601	Right mandible with I and M_2 germs
KNM-WS 12606	Lower left M_3
KNM-WS 12608	Proximal phalanx
Indeterminate hominoids	
KNM-WS 12603	Vertebral body
KNM-WS 12604	Head and neck of femur
KNM-WS 12605	Femoral head

being especially well-developed and curving smoothly round to the base. The canine crown is tetrahedral with a small apical dentine exposure; it is rotated so that its long axis lies obliquely. The maximum length is 18.3 mm and the width 13.0 mm. The canine roots are very long and converge inferiorly. The body is distinctly hollowed beneath the premolars and behind the curving canine ridge. The left maxilla contains the canine, P³ and M² crowns and the alveoli and roots of P⁴ and M¹. It reaches to the edge of the incisive foramen medially. This is at a level between the premolars. The upper canine is also long-rooted and stubby-crowned; its root defines a strong canine pillar. The zygomatic process swings abruptly laterally so that there is a deep canine fossa. The canine is 16.5 mm mesiodistally by 14.6 mm mediolaterally, with a height of 15 mm. The P⁴ is bicuspid with a large and high buccal cusp. The remaining buccal half of the M² is hardly worn, has 1.5-mm-thick enamel over the distolingual cusp and a small contact facet for M³. The maxillary sinus extends as a single cavity as far anteriorly as M¹ and hardly evaginates between the molar roots.

On the basis of several features, we believe that this material is distinct from the well-known African early Miocene *Proconsul* species⁹⁻¹². Those features which distinguish it from *Proconsul* are those which compare most favourably with *Sivapithecus*. The most obvious of these are the presence of two mandibular tori, the absence of cingula on the teeth, the strong outward rotation of the canines, the short, deep and strongly buttressed facial skeleton, the deep canine fossa and the posterior position of the incisive foramen.

On the basis of the parts known, it is difficult to offer a differential diagnosis that clearly separates the Buluk material from the genus *Sivapithecus*. A number of authorities have also included middle Miocene hominoids from Europe and Africa in this genus¹³⁻¹⁶. Unfortunately, there seems to be little consensus at present as to the exact status of the species in this genus, the hypotheses of most workers being different. Under these circumstances we believe it to be most useful to assign the large Buluk species to *Sivapithecus*. There is a close resemblance to larger specimens from India and Pakistan which have been placed in *Sivapithecus indicus*⁸ and from Turkey which have been placed in *Sivapithecus metei*¹³ or *S. indicus*¹⁷. *Sivapithecus* as presently being used by many workers almost certainly includes many widely dispersed species of early to middle Miocene hominoids which are similar in jaw and tooth morphology and which are representatives of a long-lived radiation, but whose exact cladistic relationships are not yet clear. Members of this genus were present in Africa over 17 Myr ago and persisted at least in Asia until about 7.5 Myr ago¹⁸. The Buluk species of *Sivapithecus* extends the time range of this genus back into the early Miocene. The similar and well-known *S. indicus* from the U level of the Potwar Plateau¹⁹ at close to 8 Myr is only half as old as the Buluk species. A single fragment of maxilla has previously been assigned to this genus⁹, but this specimen's provenance in East Africa is unknown. It is the type specimen of *Sivapithecus africanus*⁹ and it could be as old as 18 Myr if it is, as originally thought, from Rusinga Island. Others have suggested that the specimen came from Maboko²⁰, which would date it roughly equivalent to Fort Ternan, a site from which were obtained fragmentary specimens which have also been assigned to this genus¹⁶. As recently as 1978 this maxilla was placed in *Proconsul nyanzae* by the same authorities who now call it *Sivapithecus*²¹.

The unequivocal presence of *Sivapithecus* in Africa over 17 Myr ago also gives a new slant to the debate on hominoid origins. The presence of putative *Pongo* ancestors¹³ at 11–8 Myr in Asia was thought to be consistent with divergence dates between *Pongo* and African hominoids calculated from molecular evidence²². If there is indeed a unique ancestral relationship between the Buluk species and the later Asian ones, the fossil and molecular evidence are no longer congruent because the divergence time would be twice as great.

The recognition of *Pongo*-like features in Asian *Sivapithecus* species²² has led many authorities to place all similar fossil

species in the same clade and, by so doing, removed them from candidacy as either African ape or human ancestors. We believe this to have been premature. Leaving aside the possibility that the Buluk species represents an extinct lineage completely independent of Asian *Sivapithecus*, it is more likely in our opinion that the *Sivapithecus* morphology was present in the early ancestors of all great apes and hominids. It has become increasingly clear that is difficult to recognize primitive or derived features in these hominoids, one authority recently uniting *Sivapithecus*, *Pongo* and *Homo* in a single clade distinct from the African ape clade²³. Rather than conclude that the Buluk *Sivapithecus* represents the beginning of the *Pongo* clade in Africa over 17 Myr ago, we view it as an early species of a genus from which both African apes and hominids could be derived. The existence of such an old specimen of *Sivapithecus* does not necessarily say very much about the divergence time of the African and Asian hominoid clades.

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Age of hominoid-bearing sequence at Buluk, northern Kenya

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In northern Kenya, detrital terrigenous sediments of the Buluk Member of the Bakate Formation contain an important vertebrate fauna, which includes material from three hominoid species, one of which has been assigned to the genus *Sivapithecus* and is described elsewhere in this issue¹. Here we report K–Ar age data on feldspars from volcanic rocks in the area that show the Buluk Member sediments were deposited between 16 and something more than 17.2 Myr ago in the late early Miocene.

The area from which the fossils were recovered lies in the upper Bakate Valley, 45 km east of Ileret and Lake Turkana in Kenya (Fig. 1). The geology has been described previously^{2,3}. Briefly, the sequence dips ~6° to the south-west (Fig. 2), and

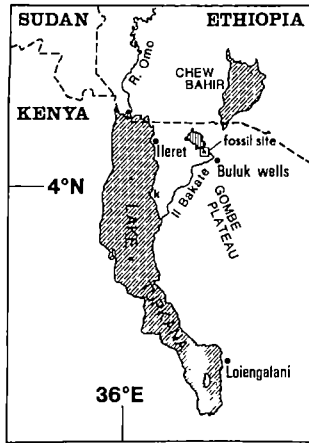


Fig. 1 Map showing location of area in northern Kenya.

consists of volcanic rocks, volcanoclastic sediments and normal detrital sediments; a composite stratigraphical section is given in Fig. 3. The Bakate Formation comprises basalts of the Irile Member, overlain by the mainly sedimentary Buluk Member, in turn overlain by basalts of the Il Jimma Member, and is succeeded disconformably by volcanoclastic sediments and ignimbrites of the Gum Dura Formation. The Buluk Member in its type section, near the hominoid locality (Fig. 2), is ~50 m thick. The lower 30 m of the Member consists predominantly of soft, poorly indurated clays, silts, sands and conglomerates of fluvial aspect. Vertebrate fossils have been recovered from sandstones, sandy siltstones and pebble conglomerates over ~15 m of the section (Fig. 3). Locally within the Buluk Member, just above the vertebrate bearing sediments, there is an essentially aphyric basalt flow ~2 m thick. The upper part of the Buluk Member, up to 20 m thick, consists of redeposited air-fall pantelleritic tuffs which are remarkably free of detrital components.

Samples for isotope dating were collected from several volcanic units in the sequence (Fig. 3) so that the reliability of the measurements could be assessed by comparison with the known stratigraphical order. Unfortunately, no suitable samples of basalt from the Irile Member were available for dating.

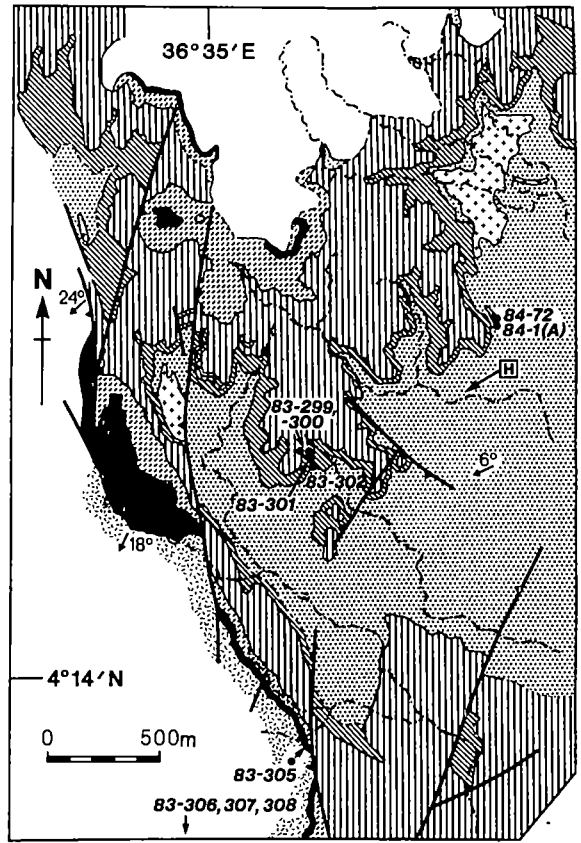


Fig. 2 Simplified geological map of the Buluk vertebrate fossil locality showing collection sites of samples used for dating (●) and of the hominoid specimens (H). Stratigraphical division and key is given in Fig. 3.

In all cases, separation of high-temperature volcanic feldspar provided suitable material for isotope dating by the conventional K-Ar method. Alkali feldspar of high purity was obtained from ignimbrite samples of the Gum Dura Formation and two tuff samples from the upper part of the Buluk Member, using

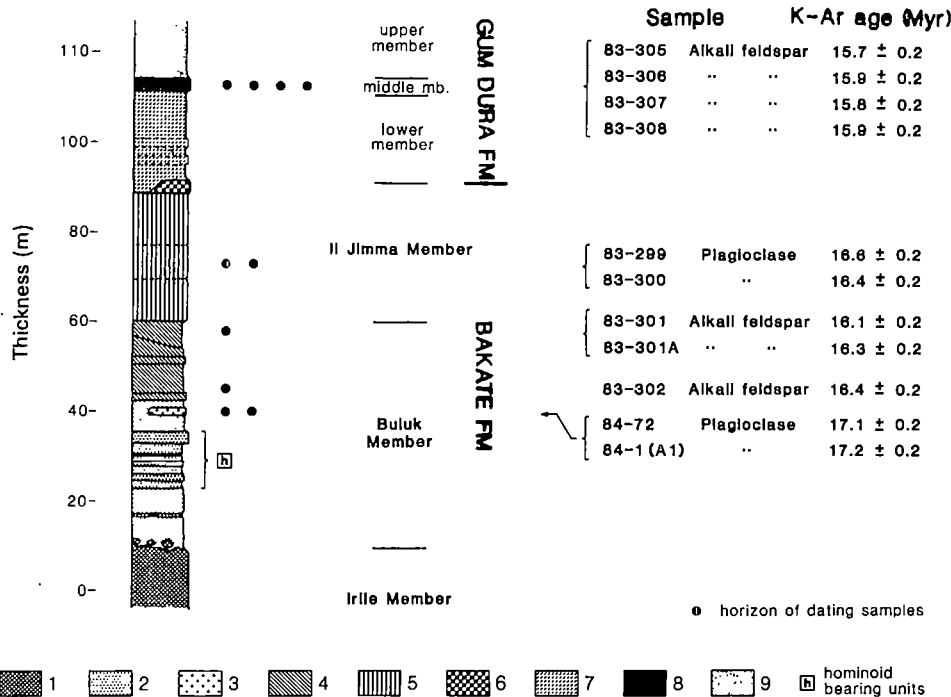


Fig. 3 Generalized stratigraphic section showing the units exposed at the Buluk fossil locality, the position of the dated samples in the sequence and their measured ages. 1, Deeply weathered porphyritic feldspar-phyric basalt lavas; 2, fluvial claystones, siltstones, sandstones and conglomerates of terrigenous origin; 3, thin aphyric or finely porphyritic basalt lava flows; 4, water redeposited air-fall pantelleritic tuffs; 5, fine-grained aphyric basalt lavas; 6, localized hydrothermal deposits of travertine and sinter; 7, pyroclastic pantellerites including air-fall deposits (largely reworked), poorly welded ignimbrites, and volcanic breccias; 8, strongly welded pantelleritic ignimbrite; 9, generally poorly welded pantelleritic ignimbrites.

Table 1 K-Ar ages on feldspars from ignimbrites, tuffs and basalts from the Buluk area, northern Kenya

Sample no.	Sample	Grid reference HBH—	K (wt %)	Radiogenic ⁴⁰ Ar (10 ⁻¹⁰ mol g ⁻¹)	100 rad. ⁴⁰ Ar	Calculated age (Myr ± 1 s.d.)	Average age (Myr ± 1 s.d.)
					Total ⁴⁰ Ar		
Ignimbrites of Gum Dura Formation							
83-305	Alkali feldspar	985677	5.795, 5.773	1.571	85.9	15.6 ± 0.2	15.7 ± 0.2
				1.594*	84.7	15.8 ± 0.2	
				1.582*	80.4	15.7 ± 0.2	
83-306	Alkali feldspar	958668	5.892, 5.910	1.627	95.5	15.8 ± 0.2	15.9 ± 0.2
				1.635*	81.2	15.9 ± 0.2	
83-307	Alkali feldspar	979656	5.862, 5.880	1.605	98.6	15.7 ± 0.2	15.8 ± 0.2
				1.629*	83.5	15.9 ± 0.2	
83-308	Alkali feldspar	982640	5.826, 5.800	1.599	97.0	15.8 ± 0.2	15.9 ± 0.2
				1.613*	80.2	15.9 ± 0.2	
Basalt, Il Jimma Member, Bakate Formation							
83-299	Plagioclase	986690	1.676, 1.686	0.487	41.2	16.6 ± 0.2	16.6 ± 0.2
				0.486	43.5	16.6 ± 0.2	
				0.488*	39.9	16.6 ± 0.2	
83-300	Plagioclase	985690	1.690, 1.685	0.483	48.3	16.4 ± 0.2	16.4 ± 0.2
				0.480*	41.5	16.3 ± 0.2	
Tuffs of Buluk Member, Bakate Formation							
83-301	Alkali feldspar	987689	6.512, 6.506	1.834	95.6	16.2 ± 0.2	16.1 ± 0.2
				1.819	92.0	16.1 ± 0.2	
				1.827*	85.5	16.1 ± 0.2	
83-301A	Alkali feldspar	987689	6.435, 6.403	1.822	84.2	16.3 ± 0.2	16.3 ± 0.2
				1.821	88.9	16.3 ± 0.2	
83-302	Alkali feldspar	987689	5.734, 5.725	1.639	90.8	16.4 ± 0.2	16.4 ± 0.2
				1.626	84.0	16.3 ± 0.2	
Basalt flow, Buluk Member, Bakate Formation							
84-72	Plagioclase	994696	1.133, 1.141	0.342	83.0	17.2 ± 0.2	17.1 ± 0.2
				0.335*	77.8	16.9 ± 0.2	
84-1 (Al)	Plagioclase	994696	1.303, 1.307	0.393	78.1	17.3 ± 0.2	17.2 ± 0.2
				0.391	80.4	17.2 ± 0.2	

$\lambda_e + \lambda_{e'} = 0.581 \times 10^{-10} \text{ yr}^{-1}$. $\lambda_{\beta} = 4.962 \times 10^{-10} \text{ yr}^{-1}$. $^{40}\text{K}/\text{K} = 1.167 \times 10^{-4} \text{ mol mol}^{-1}$.
Grid reference to East Africa Grid. Buluk 1:100,000 map, series Y633, edition 1, 1960, Survey of Kenya. Mass of sample used in Ar extraction normally 0.30 g for alkali feldspar and 0.50 to 0.70 g for plagioclase.
* Ar extraction done together with 1.0 g of essentially zero age basalt as a flux.

standard heavy liquid and magnetic separation techniques. No detrital contamination was seen in the tuffs. All the alkali feldspars were fresh and limpid, with no evidence of unmixing. Basalt samples collected from the flow within the Buluk Member and from the Il Jimma Member were essentially aphyric, although the Buluk basalt contained sparse plagioclase and altered olivine microphenocrysts. Unaltered plagioclase as thin laths, averaging 0.1–0.2 mm in length, is the most important constituent of the basalts, together with lesser amounts of pyroxene, iron oxide, some olivine and up to ~10% of green mineraloid, clay or chlorite. Because of the presence of alteration products, the samples were judged to be unsuitable for dating as whole rocks, and instead the plagioclase was separated, albeit with difficulty because of its small grain size. To obtain sufficiently good separates, the concentrates were treated briefly in dilute HF to remove mineraloid and clay in composites with the feldspar. The grain size of the plagioclase separates averaged ~50 μm. The K–Ar age measurements were made according to procedures described previously^{4,5} using flame photometry for K and isotope dilution for Ar. The Ar isotope measurements were carried out in a VG-Isotopes MM1200 mass spectrometer operated in the static mode online with a computer. As noted earlier⁵, it is difficult to extract Ar quantitatively from volcanic alkali feldspars. Each sample, contained within a Mo crucible in a water-cooled silica glass bottle attached to the high-vacuum extraction line, was held at ~1,600 °C for 40–60 minutes using radiofrequency heating. In some cases it was suspected that there was incomplete Ar recovery, despite the high temperature

of extraction. Consequently, some replicate runs were made using 1.0 g of a very young basalt as a fluxing agent with the feldspar, following the suggestion of McDowell⁶. The basalt (sample no. 83-229) used as flux contained <7.0 × 10⁻¹⁴ mol g⁻¹ radiogenic Ar. Quantitative extraction of Ar from plagioclase seems to be much less of a problem compared with alkali feldspar. Results are given in Table 1, arranged in stratigraphical order. Reproducibility of both K and Ar measurements is generally better than 1%, so that the overall precision is ~1%. The average age for each sample is reported with a minimum error of 1%, as the combined uncertainties in the calibration of the Ar tracer and K standards are at this level. Several of the earlier Ar results for alkali feldspars have been omitted from Table 1, as replicate measurements clearly showed that there had been incomplete extraction of Ar. In one case where this was suspected, the crucible was heated again at an even higher temperature (>~1,700 °C) for a further 45 min, and additional radiogenic Ar was detected, ~4% of the total radiogenic Ar. Although the four alkali feldspars separates from the Gum Dura ignimbrites averaged 1.0% higher radiogenic ⁴⁰Ar when the basalt flux was used compared with the results without the flux, we regard the agreement as satisfactory. The consistency of results from samples from the same or closely related stratigraphical levels (Table 1), together with an overall decrease in age upwards through the sequence from 17.2 ± 0.2 to 15.8 ± 0.2 Myr (Fig. 3), provides confidence that the measured ages closely reflect the time since crystallization of these volcanic rocks and the deposition of the associated detrital

sediments. However, detailed examination of the results reveals some minor inconsistencies that must be addressed.

Alkali feldspar separated from four samples of ignimbrite from the middle member of the Gum Dura Formation yield completely concordant ages, with a mean of 15.8 ± 0.2 Myr (Table 1). The four samples were collected at different localities extending as far as 4 km south of the map area; only the most northerly locality is shown in Fig. 2. The member comprises two welded ignimbrite sheets, and the fact that the samples are from the same or closely related welded ignimbrite units is supported by the concordant ages and the similarity of the K contents of the alkali feldspars. Because of the unaltered nature of the feldspars, the excellent Ar retention properties of volcanic alkali feldspar and the concordancy of the results, we are confident that the measured age is that of crystallization and cooling of the ignimbrites. Thus, 15.8 ± 0.2 Myr is considered a firm younger limit for the age of the Bakate Formation. Ages determined in this study are younger than those of 16.5 ± 0.1 and 16.6 ± 0.1 Myr (recalculated using decay constants used here), derived from $^{40}\text{Ar}/^{39}\text{Ar}$ step-heating experiments by Fitch *et al.*⁷ for alkali feldspar from the two ignimbrites from the middle member of the Gum Dura Formation elsewhere in the area.

Plagioclase from two separate samples of basalt of the Il Jimma Member, collected 50 m apart laterally and probably from the same massive flow, yielded concordant ages of 16.6 ± 0.2 and 16.4 ± 0.2 Myr (Table 1, Fig. 3). Taken at face value, these results suggest the basalt erupted 16.5 ± 0.2 Myr ago, consistent with the age determined for the overlying Gum Dura Formation. Fitch and Miller⁸ reported a K-Ar age of 17.7 ± 1.4 Myr (recalculated) on a whole rock sample of basalt from the Il Jimma Member; the large uncertainty assigned means that the age is indistinguishable from the more precise age given here.

Two separate alkali feldspar concentrates (83-301 and 83-301 A) from a coarse tuff of the Buluk Member, collected ~2 m below the Il Jimma basalt, gave ages of 16.1 ± 0.2 and 16.3 ± 0.2 Myr, respectively. Another alkali feldspar separated from a tuff ~8 m lower stratigraphically and ~2 m above the red claystones of the Buluk Member, yielded a K-Ar age of 16.4 ± 0.2 Myr (Table 1). Although the average of these ages is slightly younger than the mean age measured on the overlying Il Jimma basalts, the difference between them is small and not significant when the errors are taken into account. We conclude from these virtually concordant results that the tuffs of the upper part of the Buluk Member and the basalts of the overlying Il Jimma Member are indistinguishable in age, estimated as 16.4 ± 0.2 Myr.

Plagioclase concentrates from two separate samples of basalt from the same lava flow ~2 m thick within the terrigenous sediments of the Buluk Member, yielded concordant K-Ar ages of 17.1 ± 0.2 and 17.2 ± 0.2 Myr (Table 1, Figs 2, 3). Note that the K contents of the two concentrates differ appreciably, but that the ages agree. We believe the measured age is recording the time of crystallization and cooling of the basalt. Acceptance of this age suggests that >0.5 Myr elapsed between eruption of the basalt flow and the silicic volcanic eruptions that produced the tuffs of the upper part of the Buluk Member. The vertebrate-bearing sediments of the Buluk Member lie just below the basalt (Fig. 3), and as no significant breaks are recognized in the sequence we believe the underlying sediments are unlikely to be very much older.

Thus, the 70-m of sequence sampled yielded K-Ar ages on volcanic feldspars that are generally consistent with the stratigraphy, and in the range 17.2–15.8 Myr, covering a time span of <1.5 Myr (Fig. 3) in the late early Miocene⁹. Vertebrate bearing sediments of the Buluk Member are only a little older than the basalt dated at 17.2 ± 0.2 Myr, and probably no older than 18 Myr. The ages reported here also provide useful data for establishment of a time framework for the stratigraphical sequence found in this part of Kenya².

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Temporal hyperacuity in the electric sense of fish

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It has recently become evident that sensory thresholds for certain tasks are lower than those expected from the properties of individual receptors¹. This perceptual capacity, termed hyperacuity, reveals the impressive information-processing abilities of the central nervous system. Although much is known about spatial hyperacuity, temporal hyperacuity has received little attention². Here we demonstrate that an electric fish, *Eigenmannia*, can detect modulations in the timing (phase) of an electrical signal at least as small as 400 ns. Such sensitivity exceeds the temporal resolution of individual phase-coding afferents. This hyperacuity results from a nonlinear convergence of parallel afferent inputs to the central nervous system; subthreshold inputs from particular areas of the body surface accumulate to permit the detection of these extremely small temporal modulations.

Behavioural experiments have demonstrated that certain species of electric fish can perform remarkable analyses of the temporal structure of electrical signals^{3–5}. These animals produce an electrical signal within a species-specific frequency range via an electric organ, and they detect these signals by electroreceptors located throughout the body surface. In the context of one electrosensory behaviour, the jamming avoidance response (JAR), the fish *Eigenmannia* determines whether a neighbour's electric organ discharge (EOD), which is jamming its own signal, is higher or lower in frequency than its own. The fish then decreases or increases its frequency, respectively. To determine the sign of the frequency difference, the fish must detect the modulations in the amplitude and in the differential timing, or temporal disparity, of signals received by different regions of its body surface^{6,7}. The fish is able to shift its discharge frequency in the appropriate direction in at least 90% of all trials for temporal disparities as small as 400 ns (Fig. 1).

Intracellular electrophysiological measurements show that the phase-locked responses of even the best afferent recorded are too jittery to permit such fine temporal resolution⁸. The jitter of this time-locking can be described by its standard deviation. Even the most accurate phase-coders time-lock their spikes with a standard deviation of ~10 μ s (Fig. 2). For a sample period of 300 ms (and thus ~100 EOD cycles), which is the latency of the JAR, the 95% confidence intervals around the mean phase of occurrence of such an afferent's spikes are 2.0 μ s. Yet the fish is able to detect time disparities of several hundred nanoseconds⁸. Statistically, it would appear to be impossible for the fish, using only the information gathered from any single

Fig. 1 Percentage of correct (that is, positive) jamming avoidance responses (JARs) compared with maximal available temporal disparity. Data from three fish are presented. Inset, averaged JARs ($n=10$) for S_2/S_1 ratios of 0.1 (top) and 0.001 (bottom), corresponding to maximal temporal disparities of 80 μ s and 800 ns, respectively. Arrows indicate points at which the sign of the 4-Hz frequency difference was changed.

Methods. An immobilized fish was placed in a two-compartment chamber, and respiration by a constant flow of water through the mouth. The fish's body rested in a slot, lined with silicone grease, thus providing an electrical isolation of >40 dB between compartments (that is, a signal delivered to one compartment would be present in an adjacent compartment at a level less than 1/100th). The fish's own electric organ discharge was eliminated by intramuscular injection of 1–2% Flaxedil and replaced by a sinusoidal signal (S_1) which matched the fish's natural signal in frequency and amplitude. This EOD-replacement signal and the jamming signal (S_2) were presented through the same electrodes located in the head compartment; positive pole in the fish's mouth, negative poles in the water on either side of the fish. S_1 alone was presented in the posterior (tail) compartment; positive pole in the dorsal musculature, negative poles in the water. Thus, phase modulations existed between the signals in these two compartments. With f_1 and f_2 being the frequencies of S_1 and S_2 , a rise in the amplitude of the signal in the head compartment was accompanied by a phase advance or delay of this signal, relative to the signal in the tail compartment, when $f_2 < f_1$ or $f_2 > f_1$, respectively. Since S_1 and S_2 were delivered through the same electrodes, their amplitude ratio (S_2/S_1) was constant at all points in the chamber and equal to the ratio of the outputs of the two function generators that produced them. Thus, the degree of amplitude and phase modulations experienced by the electroreceptors in the head region could be precisely determined. The maximal time disparity, DT, is calculated from the following formula: $DT = aT/2\pi$, where T is the length of the period of S_1 , the signal used to replace the fish's electric organ discharge; S_2 is the jamming signal and a is the amplitude ratio between S_2 and S_1 . While this stimulation arrangement had the advantage of permitting exact determination of the available temporal disparity, it is a less effective means of eliciting the JAR than the multi-chamber arrangement (Fig. 3) or free field conditions (no compartments). The threshold value determined should therefore be considered a conservative estimate. JARs were measured according to the following procedure^{6,8}. The electric organ discharge frequency was determined by recording the spinal volley signal from the electric organ pacemaker via a suction electrode at the tail. This signal was amplified 1,000 times and each discharge triggered a standard square pulse that was fed into a 100-kHz computer-controlled clock for timing analysis. The fish's pacemaker (or would-be electric organ discharge) frequency was then computed. The sign of the 4-Hz frequency difference between the jamming signal (S_2) and the replacement signal (S_1) was changed every 25.6 s by the computer. Positive JAR values indicate a correct JAR (that is, the fish shifted its discharge frequency (DF) in the direction that was appropriate for the stimulus condition at the time); upward shifts for a jamming frequency that was lower and downward shifts for a jamming frequency that was higher. Shifts in frequency were integrated over the period of stimulation, and the mean shift was taken as the JAR measurement.

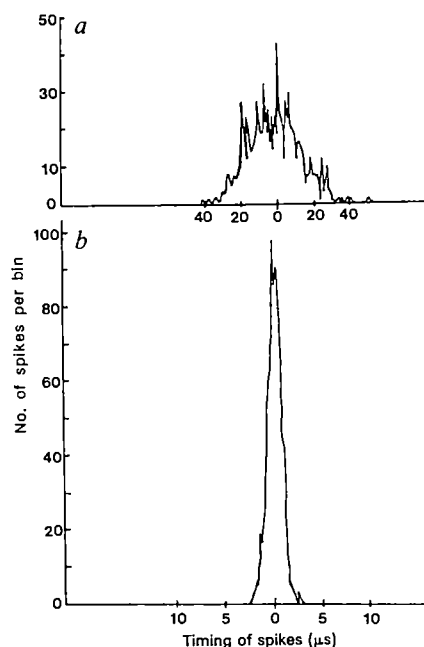
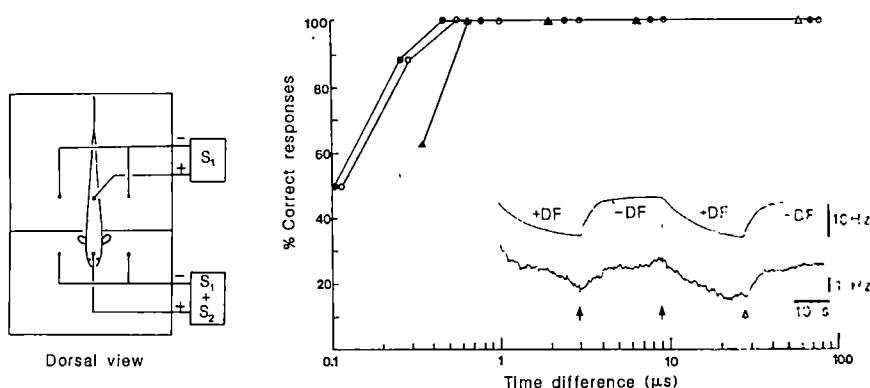


Fig. 2 *a*, Distribution of spikes around a mean time of occurrence (zero) for a single primary afferent, the most accurate phase-coder recorded. 1,024 spikes were sampled. Bin width is 0.1 μ s. *b*, Electronically generated 'spike-mimics' were recorded by the same equipment used for the neural recordings. The standard deviation of the time of occurrence of these spike-mimics, with reference to the time of their production by the waveform generator, was ~ 700 ns. The neural jitter is, therefore, not a consequence of limitations in the accuracy of measuring spike occurrences.

Methods. Spikes and spike-mimics were recorded with 3 M KCl-filled micropipettes (20 M Ω). Neural recordings were made from the anterior lateral line ganglion of anaesthetized fish. For these recordings, a sinusoidal signal, matching the fish's EOD in frequency and amplitude, was delivered through a positive electrode in the fish's mouth and a negative electrode at the tail. Forty afferents were recorded. The spike-mimic was presented through electrodes in the water. When recorded by the micropipette, these spikes had a similar rise time to that of the steepest portion of the neural spikes (1 mV μ s⁻¹). The membrane potential of the neurone was ~ 80 mV, spikes were 60 mV. Baseline voltage fluctuations were ~ 0.5 mV. This large signal-to-noise ratio reduced the temporal jitter resulting from baseline-voltage fluctuations to well below that commonly experienced in extracellular recordings. The time of occurrence of spikes with reference to each synch pulse from the signal generator, defined as the moment when the steepest portion of the signal passed a trigger level, was measured via a computer-controlled clock with a resolution of 0.1 μ s.

afferent, to reliably shift its frequency in the correct direction when the maximal temporal disparity available is only several hundred nanoseconds.

These findings lead to the prediction that the behavioural threshold should be higher when only a small group of receptors is stimulated, and that hyperacuity results from the convergence, within the central nervous system, of parallel phase-coding channels from sufficiently large areas of the body surface. These notions were tested using a paradigm which enabled us to stimulate selectively particular regions of the body surface (Fig. 3). Jamming avoidance responses could be elicited from stimulation of the body surface within any of the individual compartments, provided the ratio between the amplitude of the jamming stimulus (S_2) and the amplitude of the EOD-replacement signal (S_1) was sufficiently large. For example, an S_2/S_1

ratio of 0.05 produced a strong JAR when the stimulus was delivered exclusively to compartment 3 (Fig. 3*a*). With this ratio the fish experiences a stimulus that has a maximum relative amplitude modulation of 5% and gives rise to a maximal temporal disparity between the signal received by the left and right sides of the body of 28.4 μ s. When the S_2/S_1 ratio was reduced to 0.0016, corresponding to a maximal temporal disparity of 1.8 μ s, no JAR was observed on individual stimulation of compartments 2, 3 or 4 (Figs 3*b*, 4). Simultaneous stimulation of the body surface in these three compartments, however, elicited a strong JAR (Figs 3*c*, 4).

By electronically modulating amplitude and phase independently^{7,9}, one can demonstrate that the resolution of amplitude modulations alone is not the limiting factor in this experiment: the animal fails to increase its JAR to stimulus regimens

Fig. 3 Averaged JARs. A signal (S_1) replacing the fish's own electric organ discharge, and a jamming signal (S_2), which was alternately 4 Hz higher or lower than the replacement signal, were applied to individual compartments (a, b) or simultaneously to all three compartments (c). The amplitude ratio between these two signals, measured lateral to the fish's body, is given on the left-hand side of the figure. Centre (darkest) trace is the mean, traces to either side indicate the standard error.

Methods. An immobilized fish was placed in a multi-compartment chamber. Stimuli were delivered through wires in each of the five compartments. The EOD-replacement signal (S_1) was delivered to each compartment through an electrode in the dorsal musculature and an electrode at the bottom of the compartment, current at any given point in time thus being of the same polarity for all parts of the body surface. The 'jamming signal' (S_2) was delivered, in each chamber, through two electrodes straddling the fish. With this geometry of presentation, current flowed from outside to inside on one side of the body surface, and from inside to outside on the other side, the ventral surface being only minimally affected by the jamming stimulus. Maximal temporal disparity between the signal received by the ventral surface and that received by either lateral surface is DT (as computed according to the formula in Fig. 1), while the maximal temporal disparity between the signals received by the right and left sides is 2DT. The two stimuli to each compartment could be individually delivered or removed by means of a switching network located outside the chamber. By stimulating two adjacent sections of the body, the fish can obtain differential phase information not only by comparing the two sides of the body within the same chamber but also by comparing contralateral sides located in different chambers. No phase differences are available between ipsilateral sides in different compartments. If one assumes linear addition of inputs from separate compartments and a linear relation between the effect of a phase modulation and the size of the contralateral reference area, the simultaneous stimulation of three chambers should yield a total effect of three times the sum of the contributions from individual chambers. This is still far less than was experimentally observed (see Fig. 4). The results were qualitatively similar for all three fish tested.

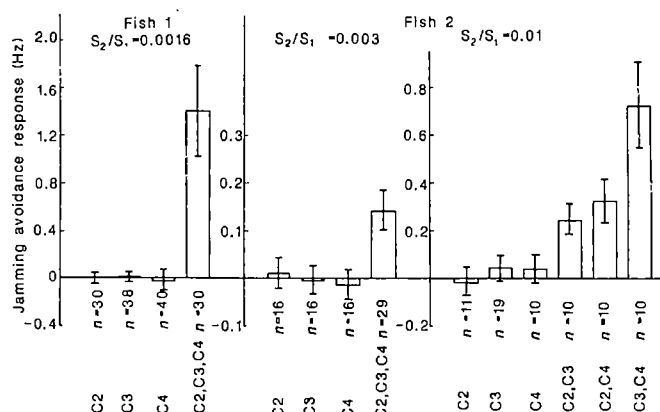
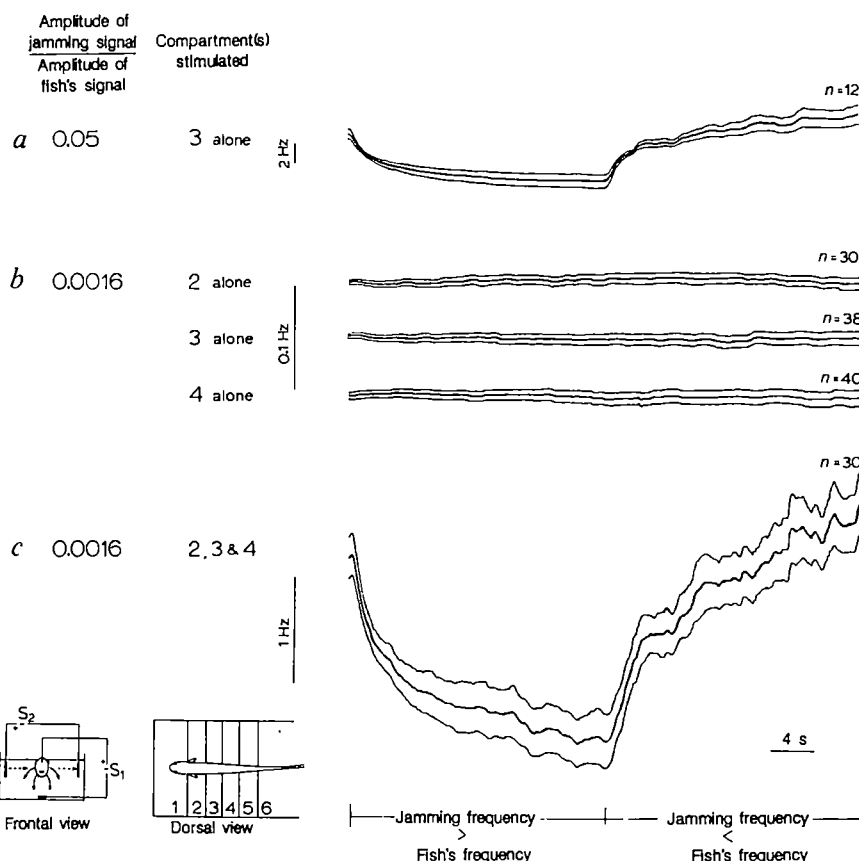


Fig. 4 Mean JAR and 99% confidence intervals for presentation of a signal (S_1) that served as a replacement for the fish's own electric organ discharge, and a jamming signal (S_2), to individual compartments (C2, C3, C4) or simultaneously to various combinations of these three compartments. The ratio between the amplitudes of these two signals is given in each case. Positive JAR values indicate that the fish shifted its electric organ discharge frequency in the appropriate direction (that is, upward when the S_2 frequency is lower than the S_1 frequency and downward for the opposite relation).

involving phase modulations at threshold level even if larger amplitude modulations are presented.

Our results indicate that electroreceptive afferent inputs converge in a nonlinear manner to achieve sensitivity to temporal disparities of several hundred nanoseconds, thus exceeding the interaural time-disparity sensitivity of owls (10 μ s; ref. 10) and of humans (15–20 μ s; ref. 11). In this process, inputs which are individually insufficient to elicit a measurable JAR (even after averaging over 2 h) produce a strong response when combined. This effect is not merely a result of the increase in total stimulus energy being presented to the fish because the amplitudes of S_1 and S_2 can be increased, while keeping their ratio constant, without altering the single-compartment response.

This temporal hyperacuity resembles hyperacuity in spatial vision in that the exquisite sensitivity is dependent on the presence of a reference point. In vision, the detection of small spatial modulations of a dot, line or edge is enhanced when a reference feature, displaced from the modulated feature, is made avail-

able¹². Similarly, for detection of small modulations in the timing of a signal, *Eigenmannia* requires a reference signal; the fish compares the timing of the signal in one region of the body surface with that in another region⁷.

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Mammalian neural crest cells participate in normal embryonic development on microinjection into post-implantation mouse embryos

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The production of chimaeric mice by aggregating pre-implantation mouse embryos¹ or by injection of cells into the blastocyst² has been of great value in analysing the regulation of early mammalian development and in dissecting the relationships of early cell lineages^{3,4}. While the totipotent cells of the pre-implantation embryo can be grown *in vitro* and thus are readily accessible to experimental manipulation, this is not possible after the embryo has implanted into the uterus. This problem has severely hampered the analysis of cell migration and of cell lineage relationships in later stages of mammalian development. In contrast, the chicken embryo can be manipulated experimentally throughout embryogenesis and this has made the bird a favourable system for studying patterns of cell migration in the development of higher vertebrates⁵⁻⁷. In mammals, the introduction of retroviruses^{8,9} and haematopoietic cells¹⁰ has provided two means of probing post-implantation development by direct intervention. I report here that cultured neural crest cells, when microinjected into 9-day-old mouse embryos, can migrate over considerable distances and participate in normal development, and the resulting chimaeric animals show pigmentation derived from the donor cells in hair and iris. The introduction of cells into post-implantation embryos may provide the means of studying patterns of cell migration in mammalian development at a level of sophistication which so far has been restricted to the chicken system.

Neural crest cells originate from the neural folds and begin migrating into many regions of the embryo at the time of neural tube closure^{5,11}. Experiments with amphibia^{12,13} and chickens^{5,11,14,15} have shown that this embryonic population of cells gives rise to an astounding variety of different cell types, including sensory and autonomic ganglia, facial bone and cartilaginous structures, stroma of various secretory glands, and the pigment cells of the skin. When neural tubes from mid-gestation mouse embryos are explanted *in vitro*, cells which resemble morphologically the well-characterized chicken neural crest cells begin to migrate from the explant¹⁶. After several days in culture, cells begin to synthesize catecholamines and become dopa-positive, indicating their differentiation into adrenergic and prepigment cells¹⁶. Pigment formation, however, has never been observed in explants of murine, in contrast to chicken neural crest cells^{17,18}. In the present study I investigated whether explanted neural crest cells would become functional melanocytes when microinjected into developing post-implantation mouse embryos.

C57BL/6J mouse embryos were isolated at day 8.5 of gestation (5–15-somite stage) and were explanted as described in Fig. 1 legend. Cells began to migrate from the explants within 24 h and the neural tubes were detached from the dishes with tungsten needles after 2 or 3 days. Figure 1 shows a typical culture at 3 days after explantation. The migrating cells have a similar morphology to that described for neural crest cells isolated from chickens¹⁹ and mice¹⁶. Cells of neural crest morphology were seen predominantly at the edges of the migrating cells, whereas in the centres of the outgrowths extensive differentiation occurred with increasing time of culture, resulting in cells of epithelial and neuronal morphology. Further characterization of the different cell types was not attempted.

For microinjection, individual cultures were trypsinized at 3–13 days after explantation, and the cells were concentrated

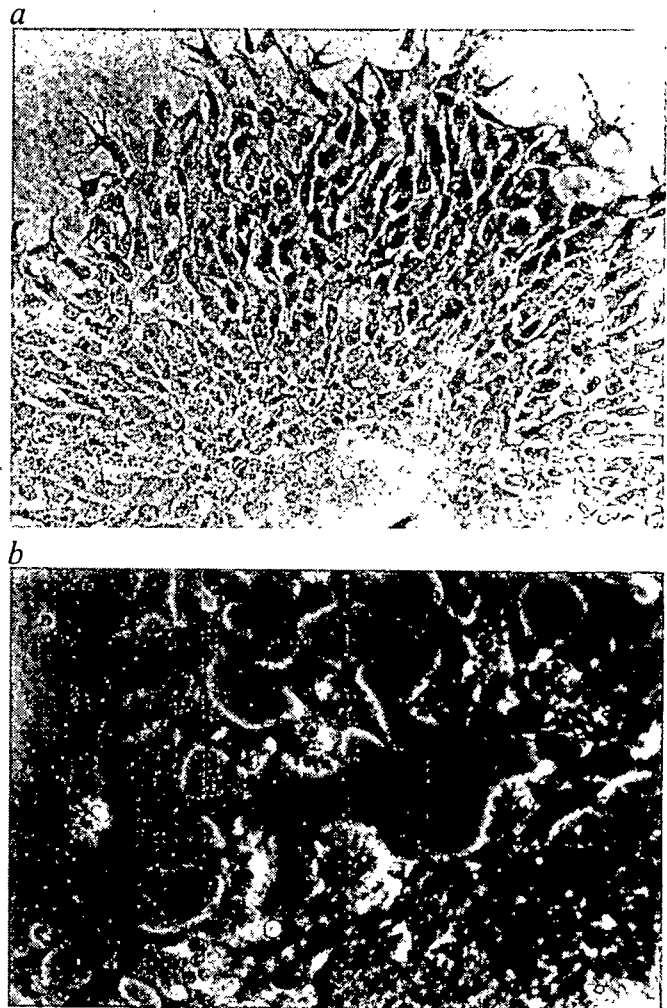


Fig. 1 *In vitro* culture of explanted neural tubes. The figure shows phase-contrast photomicrographs of cells migrating from the neural tube at 3 days after explantation. *a*, $\times 34$; *b*, $\times 112$.

Methods. C57BL/6J mouse embryos were isolated at day 8.5 of gestation (day of vaginal plug = day 0 of gestation). The embryos were freed of placenta and fetal membranes and incubated in HEPES buffer containing 1% trypsin at 5°C for 20–30 min¹⁷. Enzyme treatment was stopped by washing the embryos twice in HEPES buffer containing 10% fetal calf serum (FCS), and the neural tubes were dissected from the embryos under a dissecting microscope. The neural tubes were carefully freed of somites and all surrounding tissues and between five and eight tubes were placed in a 2-cm tissue culture dish which had been coated previously with collagen (Vitrogen) or gelatin (0.5% solution for 1 h); they were then cultured in DMEM containing 5% FCS and 5% horse serum.

by centrifugation and resuspended in 25–40 μ l of DMEM (Dulbecco's minimal essential medium) containing 10% calf serum. The total number of cells obtained per culture varied between 2×10^4 and 2×10^5 . The cells were microinjected into recipient BALB/c embryos at day 8.25, between day 8.75 and 9.25, or at day 9.75 of gestation essentially as described previously^{8,9}. Briefly, the pregnant mice were anaesthetized and laparotomy was performed by a long ventral incision. The uterus was held with a sharp forceps and cells were microinjected into individual embryos by introducing the micropipette into the ventral third of the decidual swelling. Approximately 0.1–0.5 μ l of liquid, containing 50–300 cells, was injected per embryo. Table 1 shows that approximately 60% of the injected embryos survived to adulthood.

On visual inspection of the resulting animals, I found that approximately 15% of the mice derived from embryos injected with neural crest cells between days 8.75 and 9.25 of gestation showed evidence of a pigment contribution from the donor cells

(Table 1). In contrast, none of 121 animals derived from embryos injected at day 8.25, and none of 71 animals injected at day 9.75 of gestation showed visible pigmentation. The fraction of chimaeric animals obtained, however, was strongly dependent on the age of the neural crest cell cultures. Table 2 shows that cells from 3–5-day-old cultures resulted in more than 30% chimaeric mice, while this fraction decreased to approximately 1% when cells from 11–13-day-old cultures were used for injection. This result suggests that the fraction of undifferentiated neural crest-like cells shown in Fig. 1, rather than the more differentiated cell types which accumulate with prolonged culture, give rise to pigmentation in the chimaeric mice.

Figure 2 shows typical examples of chimaeric mice. Pigmented hair was found in either the head or posterior trunk region, but never in the upper mid-trunk region or the forelimbs (see Table 3). Most chimaeras had pigmented hair in the head region which typically covered a symmetrical area extending laterally from the mid-dorsal line to both sides (Fig. 2a–c). Approximately 30% of the chimaeras were pigmented in the posterior trunk region, including the hindlimbs (7 animals; Fig. 2c, d) or the tail (3 animals; Fig. 2b). The pigmented area in the posterior trunk region frequently showed a sharp demarcation at the mid-dorsal (Fig. 2c) or mid-ventral line. However, pigmentation was not restricted to the epidermis. Microscopic inspection revealed extensive pigmentation of the iris in 10 of the 22 animals exhibiting head chimaerism. The pigment appeared to radiate in sections from the pupil to the back of the eye (Fig. 2e). Similarly, pigmented cells appeared to radiate from the optic nerve to the equator of the eye when viewed from the back (Fig. 2f). Histological examination revealed that the pigmented cells were localized in the choroid and not in the retina (U. Dräger, personal communication). This result is consistent with earlier observations indicating that the pigment of the mesodermal iris and of the choroid is derived from neural crest cells^{20,21} and not from the central nervous system (CNS), which gives rise to the pigmentation of the retina. Table 3 summarizes the types of pigment distribution seen in the 31 chimaeras. Three classes of animals were observed (types A, B and C). Pigment was restricted to the head area in 19 animals and to the posterior trunk in 9 animals. Three animals, however, exhibited pigmentation of the head as well as of the posterior trunk (type C in Table 3; compare with Fig. 2c).

The phenotype of the chimaeras indicates that neural crest cells which have been introduced into an embryo at late stages of development can participate in normal morphogenesis. How do the injected cells reach their position in the skin and iris? Because the embryo cannot be visualized at the time of injection, the pathway of donor cell migration can only be deduced from the phenotype of the chimaeras. Principally, after successful injections resulting in chimaeric mice, the cells may have been deposited either inside the embryo proper or outside it, in the extraembryonic cavities—that is, the yolk or amniotic cavity. After injection, donor cells may have migrated along aberrant pathways, for example in the trough of the exocoelomic cavity, or they may have followed normal pathways of neural crest cell migration in the dermis.

The following considerations argue against the donor cells

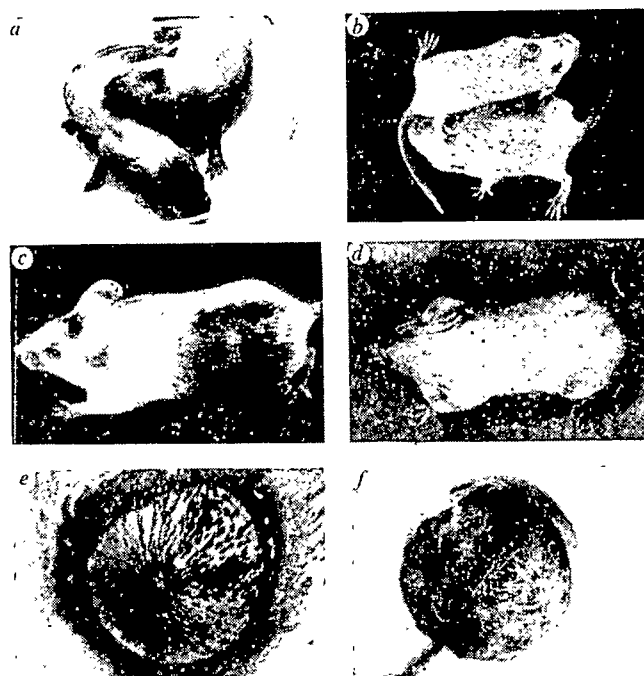


Fig. 2 Chimaeras resulting from successful injection of neural crest cells. a, Littermates (2 weeks old) with head pigmentation. b, Littermates (2 weeks old) with pigmented head and tail. c, Animal with extensive pigmentation in head, posterior trunk and hindlimbs. Note the sharp demarcation at the mid-dorsal line. d, Animal with two pigmented hindlimbs. Note the fine line of interspersed pigmented hair extending from the mid-dorsal line to both feet. e, Pigmentation in the iris. f, Pigmentation in the choroid as viewed from the back of the eye.

Methods. BALB/c mouse embryos were microinjected between days 8.75 and 9.25 of gestation with *in vitro*-cultured neural crest cells of C57BL/6J origin as described in the text and for Table 1.

having been deposited in the embryo proper or having migrated along abnormal pathways. (1) Pigmented cells always originated in the mid-dorsal line and when colonizing the head region, migrated to both sides mediolaterally, giving rise to symmetrical patches (see Fig. 2a–c). In all cases of distal pigmentation in the posterior trunk region, a fine line of interspersed pigmented hairs extending from the mid-dorsum down to the hind foot revealed the migratory path (see Fig. 2d). (2) Pigmentation was restricted to the head and posterior trunk region and was never seen in the anterior trunk or forelimbs, even in animals that were pigmented at the anterior as well as at the posterior end (see Fig. 2c, Table 3). (3) Both the radial pigmentation in the iris and choroid and the sharp demarcation of pigmentation along the mid-dorsal and mid-ventral lines seen in many of the chimaeras reveal the normal migratory pattern of neural crest cells and resemble the patterns observed in allophenic mice^{1,2,4,22}.

To explain the generation of chimaeric animals, it is reasonable to assume that the injection pipette has to penetrate the

Table 1 Results of injection of post-implantation embryos (BALB/c) with neural crest cells (C57BL/6J)

Age of embryos (days of gestation)	Injected	No. of embryos surviving to weaning	With pigment contributions
8.25	213	121 (57%)	0
8.75–9.25	340	212 (62%)	31
9.75	118	71 (60%)	0

BALB/c embryos were microinjected at day 8.25, between days 8.75 and 9.25, and at day 9.75 of gestation, with neural crest cells obtained from C57BL/6J neural tubes and cultured *in vitro* as described in Fig. 1 legend.

Table 2 Pigment contribution in BALB/c mice injected with C57BL/6J neural crest cells between days 8.75 and 9.25 of gestation

Age of neural crest culture	No. of animals	
	Total	With pigment
3–5 days	83	28
6–8 days	35	2
11–13 days	84	1

Injections with cells from 3–5-day-old cultures were performed at day 8 between 17:00 and 20:00 h, at around midnight on the same day, and between 06:00 and 08:00 h on day 9; no significant difference in the fraction of chimaeras obtained was observed. Most of the injections with cells from older cultures were performed at day 8.75.

Table 3 Pigment distribution in neural crest injection chimaeras

Type	No. of animals	Head, eyes	Pigment contribution	
			Anterior trunk and forelimbs	Posterior trunk (back, hindlimbs, tail)
A	19	+	—	—
B	9	—	—	+
C	3	+	—	+

yolk sac and probably the amnion, but not the embryo proper. The phenotype of animals of type C (Table 3) actually indicates that after a single injection the cells have migrated to both (opposite) ends of the animals while sparing the anterior and mid-trunk region. This suggests that the cells, after having been deposited in the amniotic cavity, are restricted as to where they can enter the embryo proper. The anterior and posterior neuropores, which close at the 18- and 30-somite stage respectively, and thus much later than the neural tube in the mid-trunk region, may serve as the points of entry. Having entered the embryo, the cells appear to have followed the normal pathways of neural crest cell migration from the mid-dorsal line mediolaterally, as indicated by the similarities of the pigment patterns seen in injection chimaeras to those of allophenic mice. If correct, this would indicate that neural crest cells, even when deposited outside their normal pathways of migration, can penetrate the epithelium and migrate to their normal locations in the epidermis to form functional pigment cells. The restriction of pigment to the mid-dorsum in many chimaeras may indicate, however, that the amelanotic host melanoblasts can interfere with the migration of the donor cells or with their 'homing-in' on the epidermis. Earlier observations have indeed demonstrated that amelanotic melanoblasts can prevent normal melanoblasts from entering the epidermis²³.

Only embryos injected between days 8.75 and 9.25 of gestation developed into animals with pigment chimaerism, whereas none of the embryos injected 12 h previously or 12 h later gave rise to pigmented mice. It is possible that the embryo at day 8.25 is physically too small to allow introduction of cells into the yolk sac and amnion. At day 9.75 of gestation, the neural tube is already closed at the anterior end, thus preventing the injected cells from entering the embryo at that site; the posterior neuropore may still be open, but technical difficulties rather than developmental constraints may interfere with successful colonization from that end at the later stage.

The manipulation of post-implantation mouse embryos by cell injection has great potential for the analysis of patterns of cell migration in early stages of mammalian morphogenesis under *in vivo* conditions. It would be particularly interesting to investigate whether other migratory cells such as primordial germ cells, which normally migrate from the allantois to the genital ridge between days 8 and 12 of gestation²⁴, could colonize an embryo after microinjection. The results described here suggest that the post-implantation embryo can be used to analyse cell interactions at critical stages of development. For example, cell surface antigens have been implicated as being important in cell adhesion and morphogenesis²⁵. Retroviral vectors²⁶ could be used to transfer cellular genes, such as those coding for cell surface antigens, into the cells prior to injection to elucidate the role of these antigens in cell migration and homing. This approach may allow us to study the cell-cell interactions in mammalian development that are crucial for morphogenesis and which, so far, have not been amenable to experimental analysis.

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Insulin rapidly stimulates tyrosine phosphorylation of a M_r -185,000 protein in intact cells

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Phosphotyrosine-containing proteins are minor components of normal cells^{1,2} which appear to be associated primarily with the regulation of cellular metabolism and growth^{3,4}. The insulin receptor is a tyrosine-specific protein kinase^{5,6}, and one of the earliest detectable responses to insulin binding is activation of this kinase and autophosphorylation of its β -subunit⁷⁻⁹. Tyrosine autophosphorylation activates the phosphotransferase in the β -subunit and increases its reactivity toward tyrosine phosphorylation of other substrates^{10,11}. When incubated *in vitro* with [γ -³²P]ATP and insulin, the purified insulin receptor phosphorylates various proteins on their tyrosine residues¹²⁻¹⁶. However, so far no proteins other than the insulin receptor have been identified as undergoing tyrosine phosphorylation in response to insulin in an intact cell. Here, using anti-phosphotyrosine antibodies, we have identified a novel phosphotyrosine-containing protein of relative molecular mass (M_r) 185,000 (pp185) which appears during the initial response of hepatoma cells to insulin binding. In contrast to the insulin receptor, pp185 does not adhere to wheat-germ agglutinin-agarose or bind to anti-insulin receptor antibodies. Phosphorylation of pp185 is maximal within seconds after exposure of the cells to insulin and exhibits a dose-response curve similar to that of receptor autophosphorylation, suggesting that this protein represents the endogenous substrate for the insulin receptor kinase.

To identify any phosphotyrosine-containing proteins which might appear in cells as a consequence of insulin binding, the well-differentiated and insulin-sensitive hepatoma cell line Fao^{17,18} was labelled with ³²P-orthophosphate for 2 h¹⁹. Then the cells were either treated with 100 nM insulin or left untreated and the monolayers were solubilized with Triton X-100 as described in Fig. 1 legend. Purification of the resulting extract by wheat-germ agglutinin(WGA) chromatography and immunoprecipitation with anti-insulin receptor antibody showed

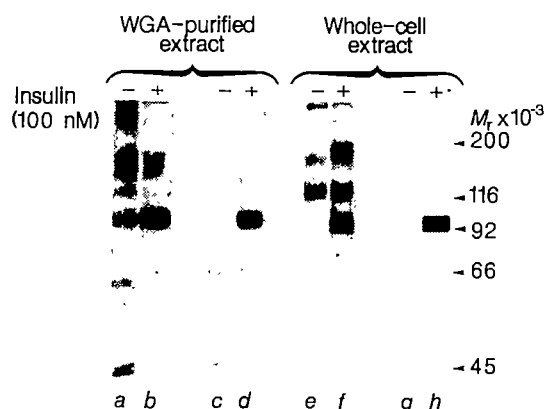


Fig. 1 Purification of phosphotyrosine-containing proteins from ^{32}P -orthophosphate-labelled Fao cells by immunoprecipitation with anti-insulin receptor and anti-phosphotyrosine antibodies. *a-d*, WGA-purified extracts immunoprecipitated with anti-receptor antibody (*a, b*) or anti-phosphotyrosine antibody (*c, d*). *e-h*, Whole-cell extracts immunoprecipitated with anti-phosphotyrosine antibody (*e, f*) or with anti-phosphotyrosine antibody, followed by anti-receptor antibody (*g, h*).

Methods. Fao cells were grown in plastic tissue culture dishes (15-cm diameter) containing 30 ml of RPMI 1640 medium (Gibco) supplemented with 10% fetal bovine serum (Gibco); 12 h before each experiment, the culture medium was changed to serum-free RPMI 1640. Confluent Fao cells were labelled for 2 h in 10 ml of phosphate-free and serum-free RPMI 1640 medium containing carrier-free ^{32}P -orthophosphate (0.5 mCi ml $^{-1}$; NEN). Cells were incubated without (-) or with (+) insulin (100 nM) at 37°C for 1 min, then the experiments were stopped quickly by removing the incubation medium and freezing the cell monolayers with liquid nitrogen. The monolayers were thawed and solubilized immediately at 4°C with 2 ml of a solution containing 50 mM HEPES pH 7.4, 1% Triton X-100, 10 mM sodium pyrophosphate, 100 mM sodium fluoride, 4 mM EDTA, 2 mM sodium vanadate, 1 mg ml $^{-1}$ aprotinin, and 2 mM phenylmethylsulphonyl fluoride. A supernatant of the whole-cell extract was prepared by scraping the cells from the dishes and sedimenting the insoluble material by centrifugation at 50,000 r.p.m. in a Beckman 70.1 Ti rotor for 60 min. WGA-purified extracts were obtained by applying the supernatant to a 0.5-cm diameter disposable column (BioRad) containing 0.2 ml of WGA-agarose (Vector). The agarose was washed with 100 ml of 50 mM HEPES pH 7.4 containing 0.1% Triton X-100, 10 mM sodium pyrophosphate, 10 mM sodium fluoride, 4 mM EDTA and 2 mM sodium vanadate, and the bound glycoproteins were eluted with two 0.5-ml portions of this wash solution containing 300 mM *N*-acetylglucosamine (Sigma). Specific immunoprecipitates were obtained from WGA-purified extracts (*a-d*) or from whole-cell extracts (*e-h*) using anti-phosphotyrosine antibodies prepared according to the method of Pang *et al.*³⁴ or anti-insulin receptor antibodies (B2) obtained from patients³¹. After incubation with the extract at 4°C for 2 h, the antibodies were immobilized on Pansorbin (Calbiochem) and the precipitates were washed three times with a solution containing 50 mM HEPES, 1% Triton X-100 and 0.1% SDS. Proteins were eluted from the phosphotyrosine-antibody complex by addition of 10 mM *p*-nitrophenylphosphate to the wash solution⁹ and from the anti-receptor antibody with SDS-polyacrylamide gel electrophoresis (PAGE) sample buffer³¹. The eluted proteins were reduced with 100 mM dithiothreitol (DTT; BioRad) and the phosphoproteins were separated by SDS-PAGE on 7.5% resolving polyacrylamide gels as described previously³². The phosphoproteins were identified by autoradiography (6 h exposure) of the stained and dried gels using Kodak X-Omat film and an intensifying screen. The WGA-purified extract was immunoprecipitated with anti-receptor antibody (*a, b*) or anti-phosphotyrosine antibody (*c, d*). The whole-cell extract was immunoprecipitated with anti-phosphotyrosine antibody and the precipitated phosphoproteins were either separated by PAGE (*e, f*) or immunoprecipitated a second time with anti-receptor antibodies before SDS-PAGE (*g, h*).

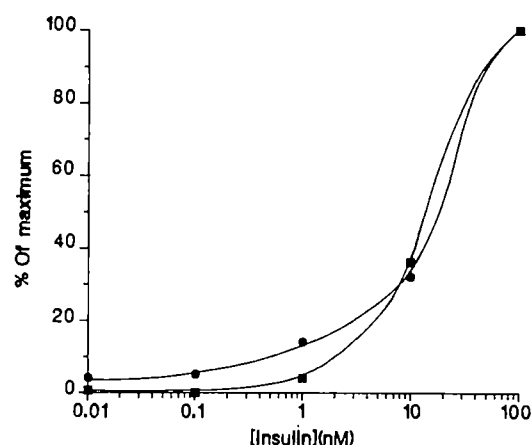


Fig. 2 Dose-response curve for effect of insulin on tyrosine phosphorylation. Labelled Fao cells were incubated in the presence of insulin for 10 min. Phosphoproteins were immunoprecipitated from the whole-cell extracts with the anti-phosphotyrosine antibody, eluted, separated by SDS-PAGE and identified by autoradiography as described in Fig. 1 legend. The intensities of pp185 (●) and the β -subunit (■) were measured by scanning densitometry and are presented as a percentage of the maximum stimulation. The maximum values, measured at 100 nM insulin, were 603 and 1,680 for pp185 and the β -subunit, respectively. The background measured in the absence of insulin has been subtracted.

a single insulin-stimulated phosphoprotein of M_r 95,000. This band was present in the basal state and increased about fourfold during insulin stimulation for 1 min (Fig. 1*a, b*). We have shown previously that this band corresponds to the β -subunit of the insulin receptor which undergoes tyrosine phosphorylation in response to insulin stimulation^{7,9,19}. When the WGA-purified extract was immunoprecipitated with a polyclonal anti-phosphotyrosine antibody a similar result was observed, except that no phosphorylation of the β -subunit was seen under basal conditions because in the intact cells the β -subunit does not contain phosphotyrosine before insulin stimulation (Fig. 1*c, d*)^{9,19}. During 1 h of incubation with insulin, the amount of ^{32}P -orthophosphate incorporated into the proteins eluted from the WGA-agarose was constant, suggesting that insulin stimulation of receptor phosphorylation does not result from a general increase in the specific activity of cellular ATP.

In an attempt to identify other phosphotyrosine-containing proteins, Fao cells were labelled and subjected to immunoprecipitation with anti-phosphotyrosine antibodies before WGA chromatography. In the absence of insulin, a single major phosphoprotein of M_r 120,000 was immunoprecipitated from the whole-cell extract (Fig. 1*e*). After incubation for 1 min with 100 nM insulin there was no change in pp120 (Fig. 1*f*), but we observed two new phosphoproteins of M_r 95,000 and 185,000 (Fig. 1*f*). pp95 is the β -subunit of the insulin receptor and was quantitatively immunoprecipitated by anti-insulin receptor serum (B2) (Fig. 1*g, h*). pp185, on the other hand was not recognized by this antibody, suggesting that it is a novel insulin-stimulated phosphotyrosine-containing protein that is antigenically distinct from the insulin receptor (Fig. 1*f, h*). The insulin dose-response curve for phosphorylation of pp185 was nearly identical to that for the β -subunit, supporting the notion that pp185 is a substrate for tyrosine phosphorylation by the insulin receptor kinase (Fig. 2).

The phosphoamino-acid composition of pp185, pp120 and the β -subunit of the insulin receptor was determined by partial acid hydrolysis and separation of the amino acids by high-voltage electrophoresis¹⁹. After insulin stimulation, each protein contained about equal amounts of phosphotyrosine and phosphoserine (Fig. 3); pp185 also contained substantial amounts

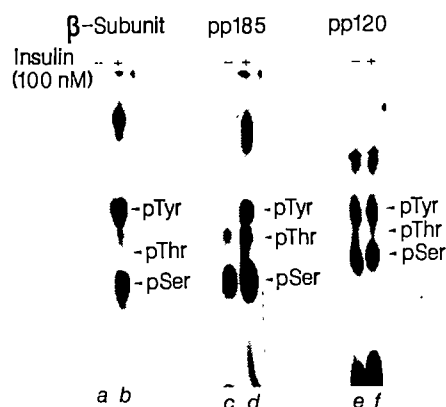


Fig. 3 Phosphoamino-acid analysis of pp185, pp120 and the β -subunit of the insulin receptor immunoprecipitated from whole-cell extracts with the anti-phosphotyrosine antibody. Fao cells were incubated in the absence (-) or presence (+) of insulin (100 nM) for 1 min. Phosphoproteins were immunoprecipitated from the whole-cell extracts with the anti-phosphotyrosine antibody and specifically eluted with *p*-nitrophenylphosphate, reduced with DTT and separated by SDS-PAGE. Dried gel fragments containing the β -subunit (a, b), pp185 (c, d) and pp120 (e, f) were incubated with trypsin ($50 \mu\text{g ml}^{-1}$) as described previously^{9,31}. The phosphoamino-acid composition of the eluted tryptic peptides was determined as described previously³¹. The phosphoamino-acid standards were visualized by reaction with ninhydrin and the radioactive amino acids were detected by autoradiography for 24 h.

of phosphothreonine. Although there was no distinct evidence of pp185 before insulin stimulation, there was a faint band that migrated slightly faster than pp185 which contained a small amount of phosphotyrosine (Figs 1e, 3c). Assuming that this is the same protein, insulin stimulated its phosphotyrosine content at least 10-fold (Fig. 3d). A significant increase in the amount of phosphoserine and phosphothreonine was also observed in pp185 after insulin stimulation, but whether this arises from *de novo* phosphorylation or tyrosine phosphorylation of previously phosphorylated pp185 is unknown. Before and after insulin stimulation, pp120 contained nearly equal amounts of phosphotyrosine, suggesting that it may be a substrate for an unidentified and constitutively activated tyrosine kinase in Fao cells.

Phosphorylation of the β -subunit is one of the earliest molecular responses that occurs in cells following insulin binding^{9,19}. As we have reported previously¹⁹, within 20 s after insulin stimulation (100 nM), phosphorylation of the β -subunit is maximal and remains at this elevated level for at least 1 h during uninterrupted exposure to insulin (Fig. 4). Phosphorylation of pp185 was also maximal by 30 s after exposure of the Fao cells to insulin, but it decreased during 60 min of continued insulin stimulation (Fig. 4); this decrease was not the result of a change in the specific activity of cellular [γ -³²P]ATP as there was no change in the level of phosphorylation of pp120 or of the β -subunit, which is rapidly dephosphorylated when the labelled ATP pool is diluted with a chase of unlabelled phosphate²⁰. Phosphoamino-acid analysis of pp185 indicated that concentrations of phosphoserine, phosphothreonine and phosphotyrosine decreased in parallel during this time interval (data not shown). These results suggest that following phosphorylation of pp185 there may be activation of a phosphoprotein phosphatase that recognizes pp185, but not the insulin receptor or pp120, as a substrate for dephosphorylation. These findings are consistent with the notion that the action of insulin may be regulated in the intact cell by both phosphorylation and dephosphorylation reactions²¹.

In the experiment shown in Fig. 4, phosphoproteins of M_r 75,000 and $>200,000$ were also detected by immunoprecipitation with the anti-phosphotyrosine antibody. The band of higher relative molecular mass showed similar kinetics to pp185,

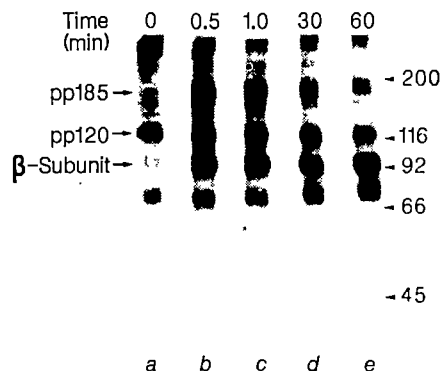


Fig. 4 The time course of insulin-stimulated tyrosine phosphorylation. Fao cells were incubated in the absence (0 min) or presence of insulin for the indicated time intervals. Phosphoproteins were immunoprecipitated from the whole-cell extracts with the anti-phosphotyrosine antibody, eluted with *p*-nitrophenylphosphate and identified by SDS-PAGE and autoradiography as described in Fig. 1 legend.

whereas pp75 was present in the basal state and tended to increase after 30–60 min of insulin stimulation. These phosphoproteins were less abundant than the others described above and were observed only after prolonged exposure of the autoradiograms. Phosphoproteins with M_r s of 300,000–200,000 were detected by anti-phosphotyrosine antibodies in human fibroblasts stimulated with platelet-derived growth factor (PDGF)²².

Like the epidermal growth factor (EGF) receptor, PDGF receptor and several oncogene products, the insulin receptor is a tyrosine-specific protein kinase. Previous studies have successfully identified several cellular substrates (pp34, pp41, pp81, enolase, lactate dehydrogenase, phosphoglycerate mutase, vinculin) for the EGF receptor^{23–26}, PDGF receptor^{22,27} and pp60^{src} (refs 23, 24, 26, 28), but so far no endogenous substrates for the insulin receptor have been identified. The present study indicates that in addition to stimulating autophosphorylation of the insulin receptor, insulin stimulates tyrosine phosphorylation of a protein in intact hepatoma cells that has a M_r of 185,000. This protein (pp185) may have gone undetected by previous studies for several reasons. First, its rapid phosphorylation and dephosphorylation in insulin-stimulated cells mean that it can be detected only at early time points. Second, WGA chromatography is often used to purify cell extracts before studying insulin receptor phosphorylation²⁹, but as pp185 does not bind to this lectin it would not be detected in such experiments. Third, sodium vanadate, an inhibitor of dephosphorylation^{19,30}, was used in our studies during cell lysis and protein purification, but it has not been used routinely in the past. Finally, two-dimensional gel electrophoresis, which has been successful in identifying many tyrosine kinase substrates^{23,28}, may miss phosphoproteins with pI values <5.5 (ref. 27) or high- M_r phosphoproteins due to the background phosphorylation that occurs in this region²². These limitations can be avoided by using anti-phosphotyrosine antibodies, which provides specific probes for studying tyrosine phosphorylation events that occur during the initial response of cells to insulin.

Although the exact nature of pp185 is unknown, we can eliminate certain possibilities. pp185 does not bind to WGA and was not immunoprecipitated by the anti-insulin receptor serum B2, which recognized the mature receptor and the glycosylated and non-glycosylated (H. A. Hedo, personal communication) forms (M_r 155,000–190,000) of the precursor^{31,32}. Furthermore, the tryptic phosphopeptide map of pp185 does not correspond to the profile of β -subunit (data not shown). Thus, pp185 is not the insulin receptor. Fao cells show no specific EGF binding and no detectable tyrosine phosphorylation during EGF stimulation (data not shown), suggesting that pp185 is not related to

the EGF receptor (M_r 175,000). pp185 is also probably not the PDGF receptor (M_r 185,000) as the latter binds to WGA^{26,33}.

Immunoprecipitation of ³⁵S-methionine-labelled Fao cells by the anti-phosphotyrosine antibody did not reveal any band at M_r 185,000, although both the α - and β -subunits were easily detected (data not shown). Thus, pp185 is possibly a minor protein that is recognized with high affinity by the insulin receptor. Whether pp185 is directly phosphorylated by the insulin receptor kinase or is the result of activation of another tyrosine kinase in response to insulin is not yet known.

The only other prominent phosphoprotein in Fao cells that is recognized by the anti-phosphotyrosine antibody has a M_r of 120,000 and is constitutively phosphorylated on tyrosine residues. The nature of this phosphoprotein is unknown. Others have reported that ATP citrate lyase (M_r 116,000) is equally immunoprecipitated with a monoclonal antibody against phenylphosphonate before and after EGF stimulation of A431 cells because it contains phosphohistidine, which cross-reacts with this antibody²⁵; however, phosphoamino-acid analysis confirmed the presence of phosphotyrosine in pp120. Insulin stimulates tyrosine phosphorylation of a M_r -120,000 protein in a Triton X-100 extract of rat hepatocyte plasma membranes purified by WGA chromatography²⁹. It is unknown whether pp120 bears any similarity to the hepatocyte protein but they are probably different as pp120 identified here does not bind to WGA.

Our results provide the first demonstration, in an intact cell, of insulin-stimulated tyrosine phosphorylation of a protein other than the insulin receptor. Identification of pp185 should elucidate the relationship between the insulin receptor kinase and the mechanism of insulin action.

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Aspirin causes short-lived inhibition of bradykinin-stimulated prostacyclin production in man

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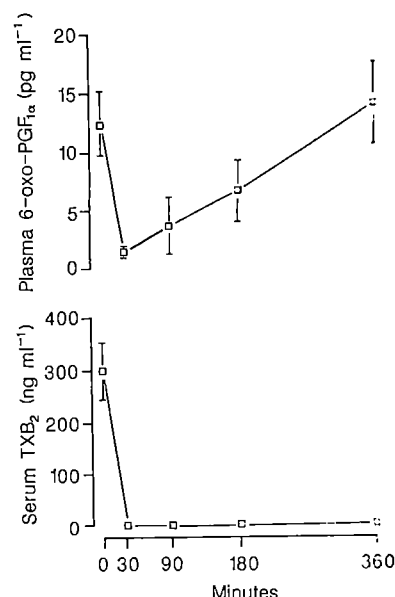
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Acetylsalicylic acid (aspirin) inhibits prostanoid synthesis^{1,2} by irreversible acetylation of fatty acid cyclooxygenase (EC 1.14.99.1)³. It thereby inhibits synthesis of pro-aggregatory thromboxane A₂ (TXA₂) by platelets^{2,4} and is widely used in the treatment and prophylaxis of vascular disease. Its efficacy, however, may be reduced since it also inhibits formation of prostacyclin (PGI₂)^{5,6} which is a vasodilator and anti-aggregatory agent^{7,8}. There is uncertainty over the optimum dose regimen for aspirin since although it inhibits platelet thromboxane production for many days⁴, the magnitude and duration of its effect on PGI₂ production by vascular endothelium *in vivo* is unknown. Resting plasma concentrations of PGI₂ (measured as the stable hydrolysis product 6-oxo-PGF_{1 α}) are at or below the limit of sensitivity of the most sensitive assays⁹ and cannot therefore be used to demonstrate a reduction in production. Bradykinin stimulates PGI₂ synthesis by cultured human vascular endothelial cells¹⁰ and we have shown that it stimulates PGI₂ production by man *in vivo*¹¹. We report here that an oral dose of aspirin (600 mg) causes rapid and substantial inhibition of bradykinin-stimulated PGI₂ production, but recovery occurs within 6 hours; this implies that endothelial PGI₂ synthesis would be spared most of the time during dosing once daily with even this relatively large dose of aspirin.

Seven healthy men aged 21-39 years, weight 68.5-85 kg, were studied. The protocol was approved by the Ethics Committee of the Royal Postgraduate Medical School, Hammersmith Hospital and subjects gave written informed consent. Bradykinin (RIA Biochemicals) was obtained as a solid, and a sterile, pyrogen-free solution in water was prepared, lyophilized and stored at -80°C. On the day of each study bradykinin was dissolved in sterile isotonic saline and passed through a 0.22- μ m filter (Millipore). PGI₂ production was stimulated by short intravenous infusions via an indwelling cannula in one forearm. Blood was sampled from a cannula in the other forearm. Bradykinin causes vasodilatation and swelling around the eyes and lips with a feeling of general discomfort, and the dose used, although the same within each subject, was varied from subject to subject depending on individual tolerance. The dose ranged from 0.15 to 0.8 μ g per kg body weight per min given as a single 5-min infusion or two consecutive 5-min infusions, the second at twice the dose of the first. The latter protocol improved tolerance in some subjects. During bradykinin infusions, blood pressure was recorded at 1-min intervals (Arteriosonde, Roche) and the electrocardiogram and skin temperature recorded continuously. Infusions were given before, and 30, 90, 180 and 360 min after drinking 600 mg soluble aspirin (Bayer) in 50 ml water. Blood samples (~20 ml) for measurement of the 6-oxo-PGF_{1 α} concentration were collected before and at the end of each bradykinin infusion. The ability of platelets to produce TXA₂ was determined by measurement of the stable hydrolysis product, TXB₂, in serum. Blood (2 ml) was taken immediately before each bradykinin infusion and incubated in a plain glass tube at 37°C for 1 h⁴. TXB₂ and 6-oxo-PGF_{1 α} were measured by a highly specific and sensitive method based on gas chromatography/negative-ion chemical ionization mass spectrometry (GC/NICIMS)^{9,12}.

Plasma concentrations of 6-oxo-PGF_{1 α} are shown in Table 1. Concentrations before bradykinin infusion were usually close to, or below, the limit of sensitivity of the assay (generally 1 pg ml⁻¹). In a few cases, especially in the control period, resting concentrations were higher, probably as a result of

Fig. 1 Effect of aspirin on serum TXB₂ concentration and bradykinin-stimulated PGI₂ production. Values shown are means \pm s.e. ($n=7$). Plasma 6-oxo-PGF_{1 α} concentrations are those measured at the end of each bradykinin infusion (Table 1). Blood (2 ml) taken immediately before each bradykinin infusion was allowed to clot in a plain glass tube at 37 °C for 1 h and serum TXB₂, the stable hydrolysis product of TXA₂, measured by GC/NICIMS. The serum TXB₂ concentration reflects the ability of platelets to synthesize TXA₂. In common with other investigators⁴, and in contrast to the effect on bradykinin-stimulated PGI₂ production, serum TXB₂ remained more than 99% inhibited 260 min after aspirin.



endothelial trauma during cannulation^{13,14}. In each subject the plasma 6-oxo-PGF_{1 α} concentration increased during the control bradykinin infusion (before aspirin), although 2 subjects (6 and 7) who tolerated only the lowest doses of bradykinin showed little stimulation. PGI₂ production was substantially inhibited 30 min after aspirin administration. In 5 of 7 subjects the stimulated 6-oxo-PGF_{1 α} concentration was below the detection limit. Assuming that the concentration in those cases was actually at the limit of detection, the mean inhibition of stimulated 6-oxo-PGF_{1 α} concentration was 86%. Recovery had usually occurred by 6 h. The serum TXB₂ concentration was inhibited by more than 99% at all times after aspirin. PGI₂ is a more potent vasodilator than bradykinin but even at 30 min after aspirin there was no attenuation of the vasodilator effects of bradykinin (data not shown). Figure 1 compares the effect of aspirin on TXA₂ and PGI₂ production. To rule out the possibility that the reduced PGI₂ synthesis at 30 min was a result of desensitization to bradykinin rather than inhibition by aspirin, three of the subjects were studied using the same protocol with placebo instead of aspirin. There was no effect on either serum TXA₂ generation or bradykinin-stimulated PGI₂ production (Fig. 2). The rapid recovery of PGI₂ after aspirin led us to believe that a dose of aspirin once daily would not cause a cumulative effect on bradykinin-stimulated PGI₂ synthesis and one subject was therefore given 600 mg daily for 7 days. On day 8, 18 h after

the last dose of aspirin, bradykinin infusion produced an increase in the plasma 6-oxo-PGF_{1 α} concentration which was again inhibited 30 min after a further dose of aspirin (600 mg) and recovered within hours (Fig. 3).

The tissue of origin of bradykinin-stimulated PGI₂ is likely to be vascular endothelium. Cultured human endothelial cells synthesize PGI₂ in response to bradykinin¹⁰, and we have found that during constant infusion of bradykinin the plasma concentration of 6-oxo-PGF_{1 α} rises to a plateau within 5 min (data not shown). Czervionke found that aspirin causes over 90% inhibition of PGI₂ production by cultured human endothelial cells, and recovery by synthesis of new cyclooxygenase is apparent within 2 h of washing¹⁵. Jaffe and Weksler obtained similar results with substantial recovery by 24 h¹⁶. The rate of recovery of cyclooxygenase activity *in vitro* after aspirin is highly dependent on culture conditions¹⁷, emphasizing the need for *in vivo* studies. Following an oral dose of 600 mg soluble aspirin, the peak plasma acetylsalicylic acid concentration occurs at 15 min, with very rapid elimination (elimination $t_{1/2}$ = 0.31 h)¹⁸. Tissues are therefore exposed to acetylsalicylic acid for only a short time. Our finding of a rapid inhibition of PGI₂ production with recovery in hours, although consistent with *in vitro* studies of endothelial cells, is seemingly at variance with the only previous *in vivo* study in man¹⁹, and with studies on human vascular tissue *ex vivo*²⁰⁻²². FitzGerald found that aspirin causes

Table 1 Effect of aspirin on bradykinin(BK)-stimulated production of 6-oxo-PGF_{1 α} (pg ml⁻¹)

Subject	Time after aspirin									
	Control		30		90		180		360	
	Pre	BK	Pre	BK	Pre	BK	Pre	BK	Pre	BK
1	1.7	12.9	<1	4.0	<1	17.1	<1	18.9	<1	26.9
2	1.1	10.2	2.2	<1	1.5	NA	<1	<1	<1	4.0
3	7.3	26.2	1.2	1.2	<1	<1	<1	3.6	<1	13.8
4	4.6	13.7	<1	<1.2	<1	1.1	<1	8.0	<1	22.0
5	<1	14.4	<1	<1	<1	<1	<1	13.0	<1	19.9
6	3.4	5.1	<1	<1	<1	<1	<1	<1	<1	8.3
7	<1	4.5	<1	<1	<1	<1	<1	<1	<1	3.3

PGI₂ production was stimulated by short intravenous infusions of bradykinin (BK). The dose of bradykinin was limited by its cardiovascular effects and adjusted for each subject on the basis of preliminary studies. Infusions were given before and at 30, 90, 180 and 360 min after a dose of soluble aspirin (600 mg in 50 ml water). Plasma concentrations of 6-oxo-PGF_{1 α} , stable hydrolysis product of PGI₂, were measured before and at the end of each bradykinin infusion by GC/NICIMS⁹. The limit of sensitivity of the assay is generally 1 pg ml⁻¹ for a 20 ml blood sample. Concentrations before bradykinin infusions were usually at or below the limit of detection. In each case there was an increase in the plasma 6-oxo-PGF_{1 α} concentration during the initial bradykinin infusion, although in subjects 6 and 7, who tolerated only the lowest doses of bradykinin, the rise was small. The increase in plasma 6-oxo-PGF_{1 α} concentration is inhibited 30 min after aspirin ($2\alpha = 0.02$, Wilcoxon's signed rank test) and recovery is seen over the following 360 min. NA, result not available.

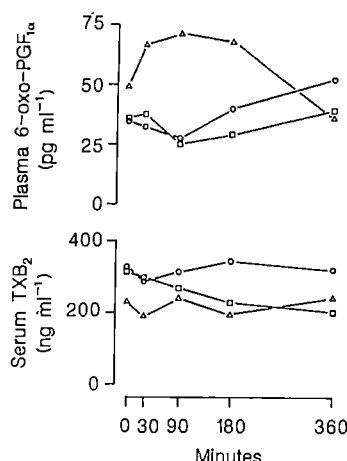


Fig. 2 Effect of aspirin placebo on serum TXB₂ concentration and bradykinin-stimulated prostacyclin production in three subjects. There was no inhibition of bradykinin-stimulated prostacyclin production, showing that the inhibition seen following aspirin is not a result of desensitization caused by the short intervals between bradykinin infusions.

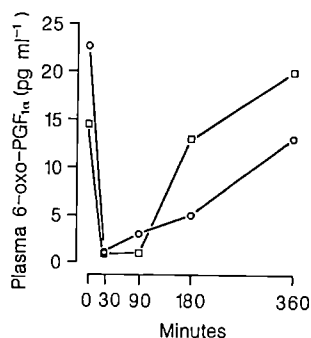


Fig. 3 Effect of chronic aspirin administration on bradykinin-stimulated PGI₂ production. One subject was studied twice. On one occasion he received 600 mg aspirin, having been drug-free for the previous 2 weeks (□), and on the second occasion he had taken aspirin 600 mg once daily for the previous 7 days, the last dose being 18 h before the dose taken on the study day (○). There was no cumulative effect of aspirin administered once daily on bradykinin-stimulated PGI₂ production, the time course of the effect being similar in the two studies. On day 8 of the chronic study, serum TXB₂ was >99% inhibited in the control (pre-aspirin) period compared with the same period of the acute study (data not shown).

only a partial (75%) inhibition of excretion of a urinary metabolite of PGI₂ even at doses of 2,600 mg daily, and recovery took more than 3 days¹⁹. The urinary metabolite, however, reflects production from many tissues with contributions from non-endothelial sources including vascular smooth muscle^{23,24}, pericardium²⁵, and gastric mucosa²⁶. If these tissues re-synthesize cyclooxygenase more slowly than does vascular endothelium, this could account for delayed recovery of the urinary PGI₂ metabolite following aspirin. If vascular endothelium contributes substantially to the urinary metabolite and recovers within hours of aspirin administration, then a 24-h urine collection will show only partial inhibition of excretion. Human vascular tissue removed 24–48 h after aspirin and incubated *ex vivo* still shows inhibition of PGI₂ production²². Much of the PGI₂ synthesized by such fragments, however, is derived from non-endothelial tissues²³, which may have a slower rate of re-synthesis of cyclooxygenase. Weksler has found that the endothelial surface of human saphenous veins has largely recovered PGI₂ synthesizing capacity by 24 h after a dose of

aspirin²⁷. Rapid endothelial cyclooxygenase synthesis could explain the finding that bovine aortic endothelial cyclooxygenase concentration is approximately 20-fold greater than that in the media²⁸.

Our results have therapeutic implications. There has been much effort expended in attempting to determine a dose of aspirin that is sufficiently low to cause selective inhibition of platelet TXA₂ synthesis without affecting PGI₂ production by vascular endothelium. The dose used in the present study is, by these standards, large. Our results indicate, however, that even this large dose has only a short-lived effect on endothelial PGI₂ production. More important than the use of a very low dose of aspirin is the need for a long dose interval, perhaps as long as several days²⁹ and certainly not less than 6 h.

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Expression of N-myc in teratocarcinoma stem cells and mouse embryos

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The N-myc gene, which is distantly related to the proto-oncogene c-myc, was first detected as an amplified sequence in human neuroblastoma cell lines and tumours^{1,2}. It has since been revealed that there is up to a 300-fold amplification of N-myc DNA in almost 50% of advanced metastatic human neuroblastomas, whereas amplification is not detected in less advanced tumours that have a better prognosis (ref. 3 and M.S., unpublished data). Although expression of N-myc is detectable in all neuroblastoma cell lines and tumours examined, its level is greatly enhanced when the N-myc gene is amplified^{4,5}. Recently, it has been shown that on co-transfection with the c-Ha-ras (EJ) gene, N-myc can induce

the malignant transformation of rat embryo fibroblasts⁶. Taken together, these data imply a function for *N-myc* in the development and/or progression of human neuroblastomas. Surveys indicate that *N-myc* also may be amplified and/or expressed in two other types of human tumours and cell lines derived from them: retinoblastomas and small cell lung cancers^{4,5,7,8}. Here, we report that *N-myc* is expressed at high levels in mouse and human teratocarcinoma stem cells, thus identifying another tumour cell type that expresses the *N-myc* gene. In addition, we found that *N-myc* is abundantly expressed in mouse embryos at mid-gestation and that its expression appears to decrease as the embryo approaches term. In the adult mouse, *N-myc* is expressed at an approximately fivefold lower level in the brain than in teratocarcinoma stem cells and embryos, and at even lower levels in the adult testis and kidney. Our data represent the first demonstration of expression of the *N-myc* gene in normal cells, and suggest that *N-myc* may be involved in mammalian embryogenesis.

Two mouse teratocarcinoma stem cell lines (embryonal carcinoma (EC) cells), PSA-1 (refs 9, 10) and F9 (ref. 11), were tested for the expression of *N-myc*. Both cell lines were isolated from embryo-derived teratocarcinomas and form tumours when injected subcutaneously (s.c.) into mice. The two cell lines are closely related to normal early embryonic cells¹² but differ in their ability to differentiate. Whereas PSA-1 cells are pluripotent and on differentiation both *in vivo* and *in vitro* give rise to a great variety of differentiated tissues, F9 cells have a very limited differentiative capacity. *N-myc* expression in PSA-1 and F9 cells was evaluated by Northern blot analysis of poly(A)⁺ cytoplasmic RNA isolated from the EC cells using a human genomic *N-myc* clone, pNb-1 (ref. 1). Both mouse EC cell lines were found to express a 3.2-kilobase (kb) *N-myc* transcript (Fig. 1). Although this transcript appeared to be relatively abundant as it was readily detected in 1 µg of total cytoplasmic RNA, it was at least 10-fold less abundant than the *N-myc* transcript of similar size observed in human neuroblastoma cells (Kelly line) in which the *N-myc* sequence is amplified ~100-fold¹ (data not shown). *N-myc* expression, equivalent to that found in PSA-1 cells, was also detected in poly(A)⁺ cytoplasmic RNA from cells of an embryonic stem cell (ESC) line (Fig. 1). Such ES cells, which are isolated from the fetal portion (inner cell mass, ICM) of a normal 4.5-day-old mouse embryo, are indistinguishable from tumour-derived teratocarcinoma stem cells and generally form teratocarcinomas with a wide variety of differentiated cell types when injected s.c. into mice^{13,14}. Thus, three tumorigenic cell lines which are known to have many properties in common with, or are directly derived from, the ICM of a normal mouse embryo, abundantly express *N-myc*. We also found that the cell line NTera2 clone D1 (ref. 15), derived from a human teratocarcinoma, abundantly expresses the 3.2-kb *N-myc* transcript (data not shown), whereas a mouse embryo fibroblast cell line, STO⁹, and a teratocarcinoma-derived endodermal cell line, PYS¹⁶, which is not tumorigenic, show no detectable *N-myc* expression (Fig. 1).

To determine whether this abundant expression in mouse EC and ES cells is due to *N-myc* gene amplification, we performed a Southern blot analysis of cellular genomic DNA (see Fig. 2). PSA-1, ES and F9 cells, in which *N-myc* is expressed at relatively high levels, appear to have the same number of copies of the gene as do mouse neuroblastoma cells, Neuro-2a, in which *N-myc* expression is very low and which have been shown to contain a single copy of *N-myc* per haploid genome⁴. Similarly, Southern blot analysis revealed equivalent numbers of *N-myc* copies in genomic DNA isolated from PSA-1, F9, ES and normal mouse spleen cells (data not shown). Therefore, the abundant expression of *N-myc* in EC and ES cells is not the consequence of gene amplification. Similarly, we found that the NTera2 clone D1 human teratocarcinoma cells, which also express relatively high levels of *N-myc* RNA, contain a single *N-myc* copy per haploid genome (data not shown).

In a recent study it was found that when human neuroblastoma cells are induced to differentiate *in vitro* they rapidly undergo a dramatic decrease in *N-myc* expression¹⁷. It seemed

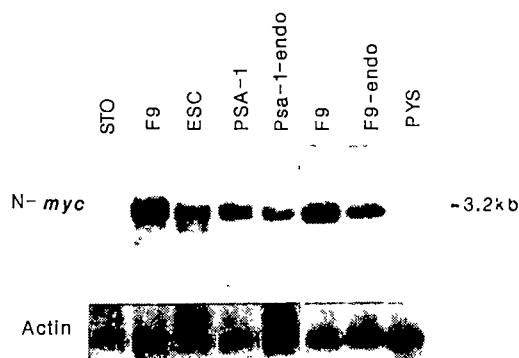


Fig. 1 *N-myc* expression in mouse teratocarcinoma stem cells and their endodermal derivatives.

Methods. STO fibroblasts, F9 embryonal carcinoma cells and PYS endodermal cells were cultured as described previously (refs 9, 11 and 16, respectively). PSA-1 and ES cells (ESC), which are maintained in the undifferentiated state by co-culture with irradiated STO fibroblastic feeders, were cultured and separated from the feeder cells¹³. Endodermal derivatives of the F9 cells (F9-endo) were obtained by incubating cultures of undifferentiated cells with 2×10^{-7} M RA and 10^{-3} M db-cAMP for 4 days¹⁸. Endodermal derivatives of the PSA-1 cells (PSA-1-endo) were obtained by allowing the PSA-1 cells to form embryoid bodies¹⁰. After 6 days of culture in bacteriological dishes, the embryoid bodies were allowed to reattach to a collagen-coated tissue culture substratum; after 2 days, the endodermal cells were collected as described previously²³. Total cytoplasmic RNA was prepared from each of the cell types after lysis of the cell membranes with Nonidet P-40, followed by phenol/chloroform extraction¹⁷. Poly(A)⁺ RNA was isolated by affinity chromatography using oligo(dT)-cellulose and each lane was loaded with ~5 µg of denatured poly(A)⁺ RNA from the designated cell type. The RNAs were separated by electrophoresis in a 1% agarose-formaldehyde gel¹⁷, and the RNA species were transferred to nylon membrane (Gene Screen; NEN), crosslinked to the nylon membrane by ultraviolet irradiation and hybridized with either the nick-translated ³²P-labelled (4×10^8 c.p.m. µg⁻¹) 1-kb *Eco*RI-*Bam*HI fragment of pNb-1 human *N-myc* DNA¹ or chick β-actin DNA (ref. 24; provided by D. Cleveland), in 1 mM EDTA, 0.5 M NaHPO₄ pH 7.2, 7% SDS, and 1% bovine serum albumin (BSA)²⁵. The blot was washed in 1 mM EDTA, 40 mM NaHPO₄ pH 7.2, 1% BSA at 65 °C. The Gene Screen membranes were stripped of hybridized probe by washing in 70% formamide, 0.2×SSC, 0.1% SDS at 65 °C for 30 min. Autoradiographic exposure time, with an intensifying screen, was ~16 h for *N-myc* and 4 h for β-actin.

possible that mouse EC cells might show a similar regulation of *N-myc* expression during differentiation to endodermal cells as no *N-myc* expression was detectable in the established endodermal cell line, PYS (Fig. 1). Relatively pure populations of endodermal cells can be derived, by different methods, from the two EC cell lines studied. When PSA-1 cells are cultured as rounded aggregates, the outer cells spontaneously differentiate to endoderm. The two-layered structures that result, consisting of a solid core of undifferentiated cells surrounded by a single layer of endoderm, are known as simple embryoid bodies because of their similarity to the fetal portion of the normal 4.5-day-old mouse embryo^{9,10}. Purified populations of PSA-1-derived endodermal cells were obtained as described in Fig. 1 legend. F9 cells can be induced to differentiate to endoderm at high frequency in monolayer culture by treatment with retinoic acid (RA) and dibutyryl cyclic AMP (db-cAMP)¹⁸. Northern blot analysis of poly(A)⁺ cytoplasmic RNA from PSA-1-derived and F9-derived endodermal cells revealed readily detectable *N-myc* transcripts (Fig. 1). Densitometer tracings of the autoradiograms shown in Fig. 1 indicate that the ratio of *N-myc* RNA to β-actin RNA is approximately two to five times lower in F9-derived and PSA-1-derived endodermal cells than in their undifferentiated progenitors. If the levels of β-actin RNA are the same in EC and endodermal cells, these data suggest that

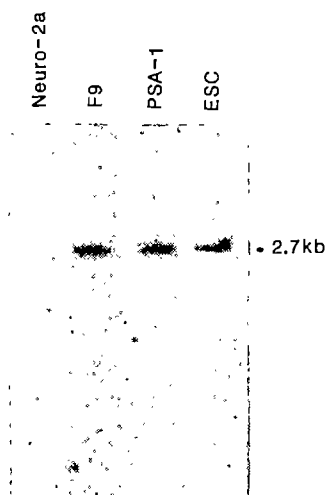


Fig. 2 Southern blot analysis of *N-myc* in genomic DNA from mouse cells. Genomic DNA was isolated from Neuro-2a mouse neuroblastoma cells⁴, PSA-1 and F9 embryonal carcinoma cells and ES cells, and 10 μ g of each was digested to completion with *Hind*III. The restriction fragments were separated on an agarose gel and transferred to nitrocellulose, then hybridized with nick-translated ³²P-labelled pNb-1 DNA as described previously¹.

the level of *N-myc* RNA decreases as EC cells differentiate to endoderm.

It is not clear why the 'primary' endodermal cells express readily detectable levels of *N-myc* RNA whereas PYS cells express little or none. It is possible that the *N-myc* expression observed in the endodermal cultures reflects the presence of a subpopulation of undifferentiated cells. However, by morphological criteria the culture of PSA-1-derived cells was almost exclusively of an endodermal phenotype. In addition, when the Northern blot of the RNAs from F9 and F9-endo cells shown in Fig. 1 was rehybridized with a probe for the *c-myc* gene, it was apparent that whereas relatively high levels of *c-myc* RNA are expressed in undifferentiated F9 cells, this RNA is barely detectable in their endodermal derivatives (data not shown). These results, which are in agreement with data from previous studies of *c-myc* expression in F9 cells and their endodermal derivatives¹⁹, suggest that there are relatively few undifferentiated cells in the endodermal populations. Another possible explanation for the differences in *N-myc* expression in primary as compared with PYS endodermal cells is that *N-myc* RNA has a relatively long half-life and that the *N-myc* RNA detected in primary endodermal cells was transcribed before differentiation. Alternatively, it is possible that the primary endodermal cultures contain cells in a state of differentiation which differs from that of PYS cells.

The results of this study raise the question of the functional significance of the abundant *N-myc* gene expression in teratocarcinoma cells. It is conceivable that the relatively high levels of *N-myc* expression in EC cells are a function of their tumorigenicity; alternatively, abundant *N-myc* expression may be a characteristic of early embryonic cells and therefore the *N-myc* expression observed in embryonal carcinoma and embryonic stem cells might be one manifestation of their close similarity to normal early embryonic cells. Although we have not tested this hypothesis directly as it is not feasible to obtain sufficient RNA for Northern blot analysis from the ICM cells to which EC and embryonic stem cells correspond, we have studied *N-myc* expression in embryos at later stages of development. Figure 3 shows the results of Northern blot analysis of *N-myc* expression in embryos collected at daily intervals from 9.5 to 17.5 days of gestation. At each stage the embryo proper was separated from the extraembryonic membranes and placenta, an appropriate number of embryos were pooled, and total cellular poly(A)⁺ RNA was isolated. In addition, total cellular

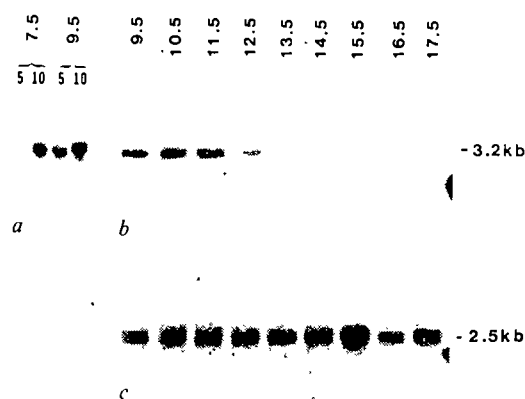


Fig. 3 *N-myc* expression in mouse embryos at various stages of gestation. *a*, Total cellular RNA isolated from embryos at 7.5 and 9.5 days of gestation and hybridized with an *N-myc* probe. The lanes contain ~5 or 10 μ g of RNA, as indicated. *b*, Poly(A)⁺ cellular RNA isolated from embryos at days 9.5–17.5 of gestation, hybridized with *N-myc* probe. Each lane contains ~5 μ g of RNA. *c*, The blot shown in *b* was stripped of probe as described in Fig. 1 legend and hybridized with a subclone of exon 3 of the human *c-myc* oncogene (*Cla*I/*Eco*RI fragment; provided by N. Hay). **Methods.** Embryos were obtained by mating mice of various inbred genotypes. The day on which the genital plug was detected was designated as day 0.5 of gestation. At each stage, total RNA was immediately extracted from a pool of embryos following homogenization in guanidine thiocyanate²⁶. RNA was isolated and analysed as described in Fig. 1 legend.

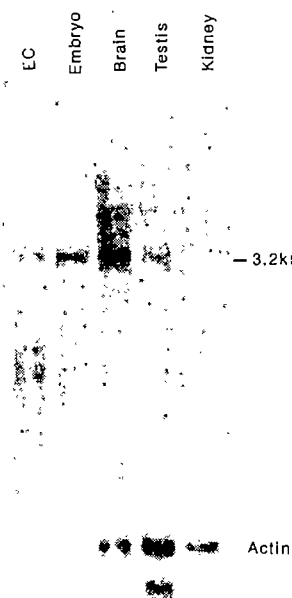


Fig. 4 *N-myc* expression in adult mouse organs. Approximately 4 μ g of poly(A)⁺ RNA from PSA-1 teratocarcinoma stem cells (EC) and from embryos at day 11.5 of gestation, and ~10 μ g of poly(A)⁺ RNA isolated from organs obtained from randomly bred adult mice, were analysed as described in Fig. 1 legend.

RNA was isolated from a pool of embryos at 7.5 days of gestation. The results of this experiment show that *N-myc* RNA was detectable at all stages tested. Figure 3*a* shows that *N-myc* RNA is detectable in 5 μ g of total RNA from embryos at 7.5 and 9.5 days of gestation, indicating that *N-myc* expression is relatively abundant at these stages of development. The level of expression in these embryos is roughly equivalent to that in PSA-1 cells (data not shown). Figure 3*b* shows the level of *N-myc* expression in poly(A)⁺ RNA isolated from embryos at days 9.5–17.5 of gestation. Roughly equal amounts of RNA were loaded in each lane, and this equivalence was confirmed by

rehybridization of the blot with a cloned probe for β -actin (data not shown). From these results it seems that the level of N-myc RNA is highest at days 7.5–11.5 of development and thereafter decreases with increasing time of gestation. The Northern blot shown in Fig. 3b was also rehybridized with a probe for the c-myc gene (Fig. 3c); in contrast to the differential expression observed for N-myc, the levels of c-myc RNA did not change with increasing stage of gestation. The latter results are largely consistent with previous studies²⁰. These data, taken together with the results of studies of the expression of various other oncogenes in mouse embryos^{20–22}, indicate that N-myc, c-myc and other oncogenes are regulated independently during development.

We also tested various organs from adult mice for expression of N-myc. N-myc RNA was readily detected in poly(A)⁺ RNA isolated from brain, was less abundant in RNA from testis and kidney (Fig. 4), but was not detectable in RNA from spleen or liver (data not shown). Densitometer tracings of the autoradiograms indicated that the ratio of N-myc to β -actin RNA is approximately fivefold higher in teratocarcinoma stem cells and in embryos at the mid-gestation stages of development than in brain.

The data presented here demonstrate that the N-myc gene is expressed during normal mouse embryogenesis and in certain organs of the adult. From the results of our study of teratocarcinoma cells, and assuming that these cells provide a valid model system for the study of gene expression in the peri-implantation mouse embryo, it seems likely that N-myc is expressed at relatively high levels by ICM cells at around the time of implantation. In contrast, primary endoderm, which is the first differentiated cell type formed by the ICM, may express it at a lower level. We have demonstrated directly that abundant N-myc expression is characteristic of the mid-gestation embryo and that the level of expression decreases at later stages of embryogenesis. An intriguing possibility is that the abundant N-myc RNA detected in embryos at earlier stages of development is a manifestation of N-myc expression in most or all cells of the embryo, whereas the decreased levels of N-myc RNA at later stages reflect expression in a very limited number of cell types, possibly only in those tissues in which N-myc is expressed in the adult (for example, brain, testis and kidney). The results of *in situ* hybridization studies of embryos and adult organs, now in progress, will help to elucidate the tissue and cell specificity of N-myc expression. The information from such studies should ultimately contribute to our understanding of the normal function of the gene.

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Isolation, sequence determination and expression in *Escherichia coli* of the isopenicillin N synthetase gene from *Cephalosporium acremonium*

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The enzyme isopenicillin N synthetase (IPS) catalyses the oxidative condensation of δ -(L- α -aminoadipyl)-L-cysteinyl-D-valine (LLD-ACV) to isopenicillin N, which is a central reaction in the pathway to clinically important penicillins and cephalosporins. Here we report the cloning, characterization and expression in *Escherichia coli* of the gene encoding the IPS protein in *Cephalosporium acremonium*. The IPS gene was identified by purifying IPS protein, determining the first 23 amino-terminal amino acids, preparing a set of synthetic oligonucleotides encoding a portion of the determined amino-acid sequence, and probing a cosmid genome library with the mixed oligonucleotides. A cosmid hybridizing with the probe was isolated and the IPS gene was localized and sequenced. The IPS gene encodes a polypeptide of relative molecular mass (*M*_r) 38,416. When this open reading frame was cloned into an *E. coli* expression vector and inserted into *E. coli*, the recombinant *E. coli* produced a new protein co-migrating with authentic IPS as the major protein of the cell (~20% of cell protein). Crude cell extracts condensed LLD-ACV to a penicillinase-sensitive molecule whose antibacterial activity indicated that it was isopenicillin N.

The conversion of LLD-ACV to isopenicillin N by IPS or cyclase is carried out by a variety of organisms, including *Penicillium chrysogenum*, *C. acremonium*, *Streptomyces clavuligerus* and other Actinomycetes. Penicillins and cephalosporins are isolated from these organisms for the manufacture of important β -lactam antibiotics. The cyclase enzyme, in the presence of Fe²⁺ and ascorbate, removes four hydrogens from LLD-ACV and consumes one oxygen, resulting in the formation of the two rings found in isopenicillin N¹. Most of the studies of the IPS reaction have examined enzymes from *C. acremonium* and *S. clavuligerus*. The *C. acremonium* enzyme has been purified^{2,3}, and used to condense not only LLD-ACV to isopenicillin N, but also variants of LLD-ACV to different β -lactam structures^{4–7}. In order to extend our knowledge of the properties and potential of the IPS enzyme, we have cloned the IPS gene and expressed the activity in *E. coli*.

IPS protein from *C. acremonium* was purified using modified published procedures² with additional reverse-phase HPLC steps. The purified protein was then subjected to amino-terminal sequence analysis, and 23 amino-acid residues were determined (see Fig. 1). Sixty-four synthetic oligonucleotides were prepared, one of which must be the perfect match of the coding information

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Fig. 1 Amino-terminal sequence analysis of IPS and synthesis of oligomeric DNA probes. **A**, The first 23 amino-terminal amino-acid residues of IPS. The question mark indicates that the first cycle of the sequenator run was ambiguous. The underlined sequence (residues 18–23) represents the region encoded by the synthetic oligonucleotides. **B**, The possible RNA codons for the underlined sequence in **A**. As the third position of each codon is variable in this region, all possibilities are shown. **C**, Two sets of 17-mer oligonucleotides incorporating the possibilities shown in **B** were synthesized. Each pool has a complexity of 32. **D**, The experimentally derived DNA sequence and the corresponding predicted amino-acid sequence (see Fig. 3).

Methods. Extraction and purification of IPS from *C. acremonium* were done as described by Pang *et al.*² except that the Dyno-Mill agitator disks were run at 2,000 r.p.m. to keep the temperature below 5°C during disintegration of the mycelium. The peak fractions containing the synthetase were used for further processing. Nearly pure IPS protein was applied to a ProRPC HR 5/10 C₈ reverse-phase column using a Pharmacia FPLC system. The A solvent was 0.1% trifluoroacetic acid and 0.1% morpholine in water; the B solvent was 80% isopropanol, 20% acetonitrile with 0.1 ml per 100 ml each of trifluoroacetic acid and morpholine. The column was run at 0.3 ml min⁻¹ with a series of the following linear gradients: from 0 to 5 ml of elution buffer, B went from 0 to 30%; from 5 to 23 ml B went from 30 to 70%; and from 23 to 25 ml B went from 70 to 100%. The 64 deoxyoligonucleotides were chemically synthesized in two pools of 32 17-mers each, using an Applied Biosystem DNA Synthesizer (Model 380-A) according to the manufacturer's recommended protocols. Oligomers were purified by 20% polyacrylamide/7 M urea gel electrophoresis.

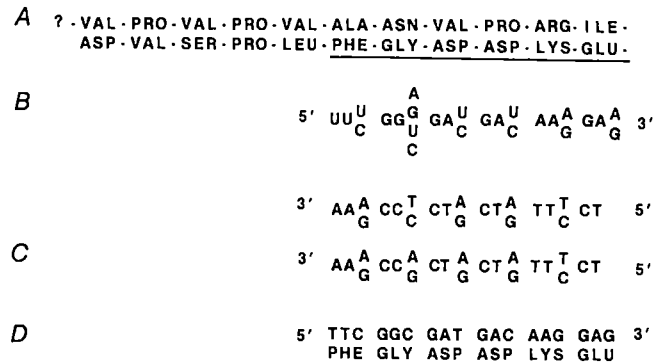
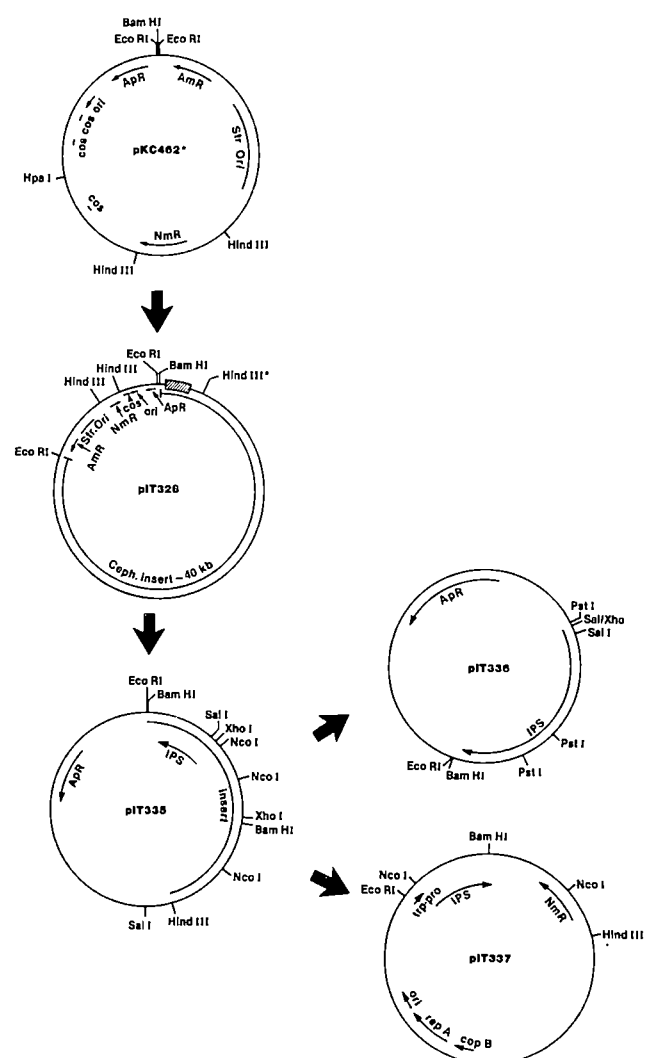


Fig. 2 Schematic diagrams of cosmids and plasmids used in the present study. The size of the circle is not proportional to the size of the plasmid. Except where indicated below, if a restriction enzyme cut site is indicated on the drawing, all restriction sites for that enzyme are included in the diagram. pKC462*, a 12.4-kb cosmid constructed by R. N. Rao and R. Stanzak (personal communication) which contains a *Streptomyces* origin of replication (Str Ori) from pFJ103 (ref. 10), and an *E. coli* origin of replication (ori) from pBR322. Antibiotic resistance markers on this plasmid are the ampicillin resistance gene (ApR) from pBR322, the apramycin resistance gene (AmR) from pKC222 (ref. 11), and the neomycin resistance gene (NmR) from Tn5. The three *cos* sites (*cos*) are from bacteriophage λ . pKC462* contains a unique *Bam*HI restriction site (used in this study for cloning) flanked by unique *Eco*RI restriction sites. pIT328, an ~50-kb plasmid created by ligating *C. acremonium* genomic DNA partially cleaved with restriction enzyme *Mbo*I into the *Bam*HI site of pKC462*. pIT328 contains ~40 kb of *C. acremonium* DNA (Ceph. insert). The cross-hatched box indicates the protein-coding region of the IPS gene. *Hind*III* is one of at least 10 *Hind*III sites in the insert, the rest of which are not shown. pIT335, an 8.2-kb plasmid, was constructed by digesting pIT328 with *Hind*III and re-ligating, thus eliminating half the vector and all but ~4.5 kb of the *Cephalosporium* DNA insert. The IPS gene is transcribed in the direction of the arrow. Only the relevant restriction sites are shown, and one more unmapped *Nco*I restriction site exists outside the IPS gene in pIT335. pIT336, a 4.1-kb plasmid, was created by cloning a 1,400-bp *Eco*RI-*Xho*I fragment from pIT335 into the *Eco*RI-*Sal*I sites of pUC8 (ref. 12). The *Cephalosporium* DNA insert (IPS) contains most of the protein-coding region of the IPS gene. pIT337 is an 11.6-kb expression plasmid containing the complete coding region of the IPS gene. The 1,460-bp *Nco*I-*Bam*HI fragment from pIT335 was ligated with two fragments (an 8.7-kb *Nco*I-*Nco*I fragment and a 1.5-kb *Nco*I-*Bam*HI fragment) from pCZ106. pCZ106 is a derivative of pCZ115 (ref. 13) which contains the bovine growth hormone gene transcribed from the *E. coli* tryptophan promoter and a temperature-sensitive copy control (runaway replicon) system. The *E. coli* tryptophan promoter (*trp* pro) drives transcription of the IPS gene. The location and direction of transcription of the IPS gene and of the neomycin resistance gene (*NmR*) are also indicated.

Methods. *C. acremonium* genomic DNA was isolated from protoplasts prepared according to Queener *et al.*¹⁴. The protoplasts were lysed by incubating them in 3% *N*-lauroyl sarkosyl, 0.5 M Tris-HCl pH 9.0, 0.2 M EDTA at 65°C for 15 min. The mixture was cooled and then extracted with buffered phenol (10 min) and chloroform (10 min). The DNA layer was mixed with a 1:10 volume of 5 M KOAc and spooled with 2 vol. of cold 100% ethanol. DNA was dissolved in 2 ml TE buffer (10 mM Tris-HCl pH 7.5, 0.1 mM EDTA) then treated with 50 μ g ml⁻¹ proteinase K (Sigma; Type XI) at 65°C for 1 h in 0.01 M Tris-HCl pH 7.8, 5 mM EDTA, 0.5% SDS. The solution was then extracted for 10 min each with buffered phenol and chloroform and the DNA respoiled and suspended in 0.5 ml TE buffer. Partial enzymatic digestions with *Mbo*I were done to achieve an average size of 30–40 kb. pKC462* was digested with *Hpa*I, treated with alkaline phosphatase, then cut with *Bam*HI. The partially digested genomic DNA was ligated into the *Bam*HI site of pKC462* and the recombinant DNA molecules were packaged into phage λ heads using Promega Biotec packaging extracts according to the recommendations of the manufacturer. *E. coli* SF8 cells grown in the presence of 0.2% maltose were transduced with the recombinant λ particles and cells containing cosmids were selected on plates containing 100 μ g ml⁻¹ ampicillin and then grown in liquid culture in microtitre dishes in the presence of 100 μ g ml⁻¹ apramycin. The library was screened according to the method of Grunstein and Hogness¹⁵. Oligonucleotide probes (described in Fig. 1 legend) were kinased under conditions described previously¹⁶. The nitrocellulose filters containing the recombinant *E. coli* were hybridized at 42°C for 2 h in 10 \times Denhardt's, 6 \times SSC, 0.1% sodium pyrophosphate¹⁷. Washes were at room temperature in 6 \times SSC for 30 min, followed by another 5-min wash in 6 \times SSC at 42–58°C.



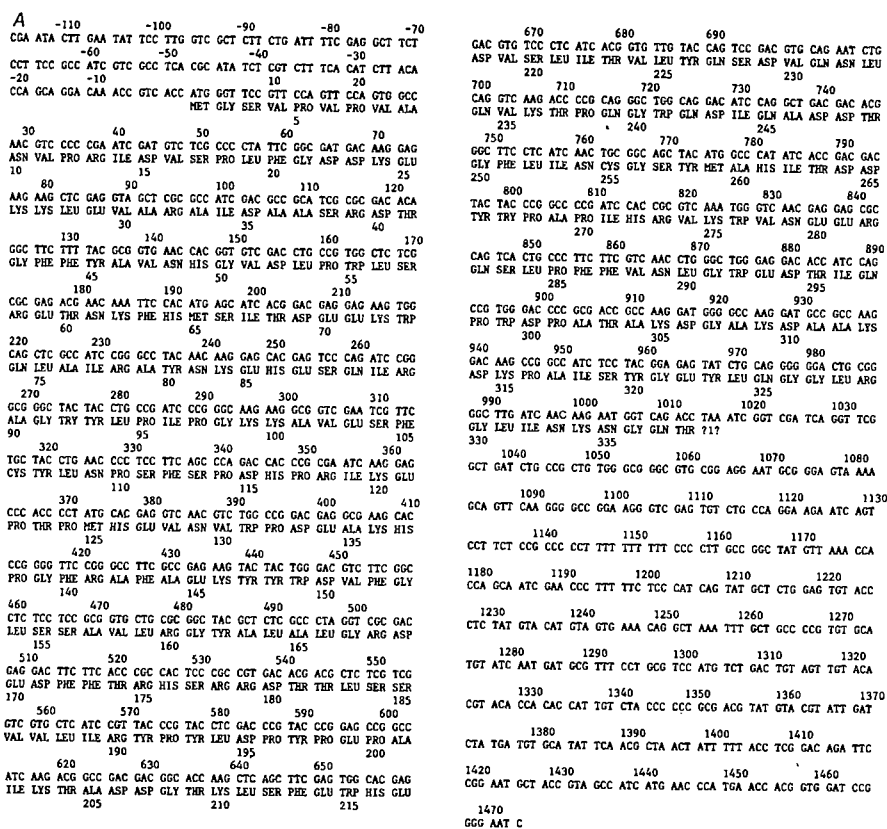


Fig. 3 A, Complete nucleotide and predicted amino-acid sequence of isopenicillin N synthetase gene from pIT335. The DNA sequence was determined by the chemical method of Maxam and Gilbert⁸ as described previously¹⁶. Both strands were sequenced for over 95% of the protein-coding region, and the remaining areas were sequenced at least twice from different sites on the same strand. The primary sequence of the IPS gene, together with 118 bp preceding the initiation codon and 455 bp after the termination codon, is shown with the predicted amino-acid sequence of a 1,017-bp open reading frame. Numbers above the nucleotide sequence refer to base-pair location. Amino-acid residues are numbered below the protein sequence. The proposed translation initiation site is amino-acid 1. B, Analysis of G+C content as a function of position in the codon. Each curve represents the G+C content of one of the three codon positions. The per cent G+C at each base is calculated as a forward average of 20 codons according to the algorithm of Bibb *et al.*⁹. For clarity, the curves presented are smoothed slightly relative to the original computer print-out. The heavy line corresponds to the first base of a codon, the dotted line refers to the second base, and the thin line refers to the third position. The arrows along the axis indicate the limits of the IPS protein-coding region (see A).

for amino acids 18–23 (Phe-Gly-Asp-Asp-Lys-Glu) encoded by the IPS gene. The 64 possible 17-mer oligonucleotides were synthesized in two pools of 32 (Fig. 1). The pool of 32 that hybridized most efficiently to restriction enzyme-cleaved *C. acremonium* genomic DNA immobilized on nitrocellulose was used for further experiments.

The synthetic 17-mer oligonucleotides were used to screen a cosmid genome bank for the IPS gene. The region of one cosmid, pIT328, which hybridized to the oligonucleotide probes was determined to be adjacent to the *E. coli* selectable markers by restriction mapping and Southern hybridizations, so the cosmid was truncated by restriction enzyme *HindIII* digestion and re-ligation to yield the 11-kilobase (kb) plasmid pIT335 (Fig. 2). The DNA sequence of a portion of pIT335 was determined by the Maxam-Gilbert technique⁸ and the predicted amino-acid sequence of one region matched the experimentally determined amino-acid sequence of the IPS protein (Fig. 3). The open reading frame containing this information (Fig. 3) can encode a polypeptide of M_r 38,416. The reported M_r of the IPS protein, as determined by SDS-polyacrylamide gel electrophoresis, is 40,000 (ref. 3) (see also Fig. 4).

The predicted amino-acid sequence of the IPS protein from pIT337 (Fig. 3) begins with methionine and glycine residues which are not found in the protein isolated from *Cephalosporium* cells. These residues are evidently cleaved post-translationally in *Cephalosporium*.

The protein-coding region of the IPS gene consisted of 63% G-C base pairs. Because of the high G+C content, we used an algorithm described by Bibb *et al.*⁹ to examine the G+C content of the DNA sequence as a function of position in the triplet codons. Bibb *et al.* have shown that the G+C content of the

positions in the triplet codons is biased within protein-coding regions but relatively random outside protein-coding regions for organisms with DNA of high G+C content. In particular, Bibb *et al.* found that the G+C content of the first position in the codon was about average, while that of the second position was much lower, and that of the third position much higher than average. As shown in Fig. 3, the same pattern of G+C content bias was observed for the open reading frame encoding the IPS protein. This provides further evidence that the open reading frame identified by sequencing is correct for its entire length, since a shift in reading frame would have also shifted the G+C content bias.

To further prove that the open reading frame encodes the IPS protein, we fused the open reading frame to an *E. coli* expression system. The open reading frame from pIT335 was isolated on an *NcoI*-*BamHI* fragment. The recognition sequence for the restriction enzyme *NcoI*, 5'-CCATGG, surrounds the methionine codon that initiates translation of the open reading frame (Fig. 3). The restriction enzyme *BamHI* cleaves ~450 base pairs (bp) downstream from the proposed translation termination site. The *NcoI*-*BamHI* fragment was ligated into an *E. coli* expression vector to create pIT337. The *E. coli* expression vector used the promoter and ribosome binding site from the *E. coli* tryptophan operon and a temperature-sensitive copy control system ('runaway replicon'). *E. coli* cells transformed with pIT337 produce a new protein which co-migrates with authentic IPS protein on SDS-polyacrylamide gels. At low temperatures the cells produce low amounts of the new protein, but at high temperatures they produce about 20% of total cell protein as the putative IPS polypeptide (Fig. 4). The other new major protein band produced by the transformed *E. coli* cells is

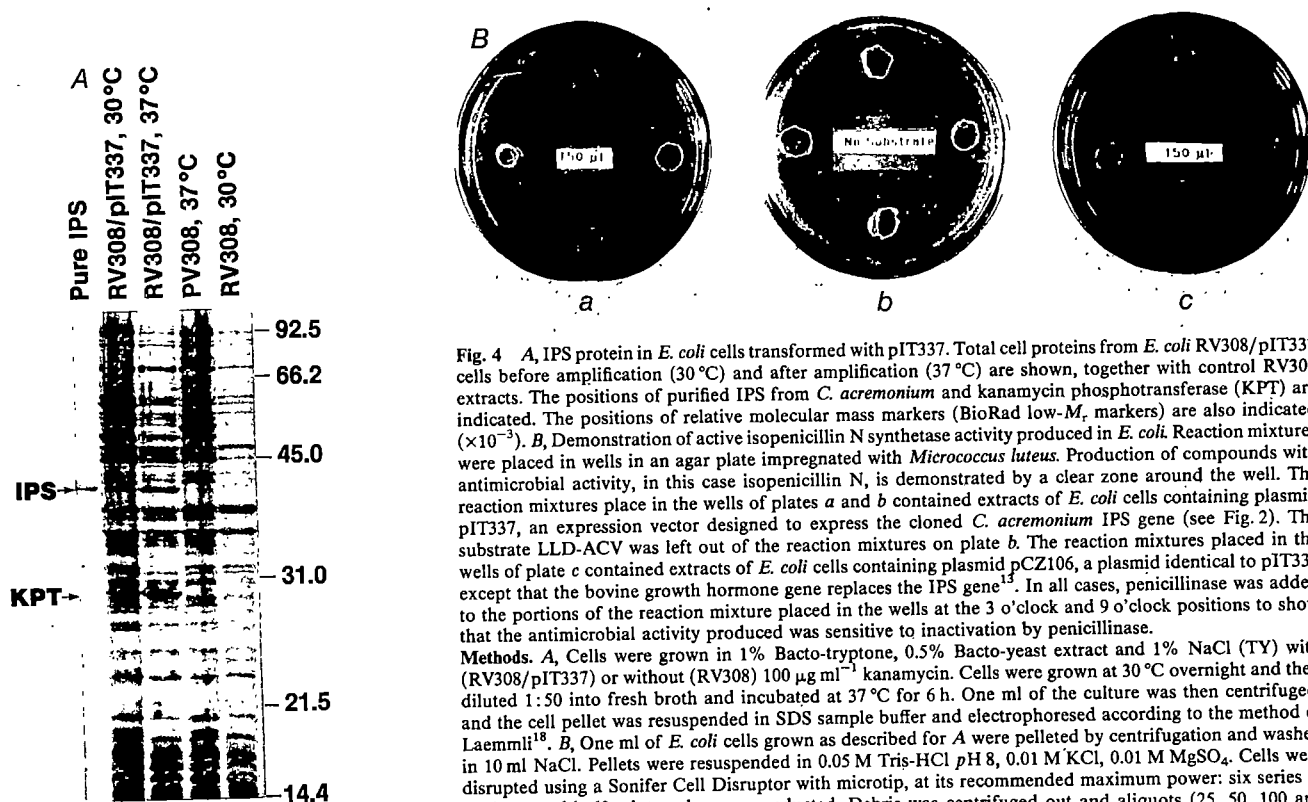


Fig. 4 A, IPS protein in *E. coli* cells transformed with pIT337. Total cell proteins from *E. coli* RV308/pIT337 cells before amplification (30 °C) and after amplification (37 °C) are shown, together with control RV308 extracts. The positions of purified IPS from *C. acremonium* and kanamycin phosphotransferase (KPT) are indicated. The positions of relative molecular mass markers (BioRad low-M_r markers) are also indicated ($\times 10^{-3}$). B, Demonstration of active isopenicillin N synthetase activity produced in *E. coli*. Reaction mixtures were placed in wells in an agar plate impregnated with *Micrococcus luteus*. Production of compounds with antimicrobial activity, in this case isopenicillin N, is demonstrated by a clear zone around the well. The reaction mixtures placed in the wells of plates a and b contained extracts of *E. coli* cells containing plasmid pIT337, an expression vector designed to express the cloned *C. acremonium* IPS gene (see Fig. 2). The substrate LLD-ACV was left out of the reaction mixtures on plate b. The reaction mixtures placed in the wells of plate c contained extracts of *E. coli* cells containing plasmid pCZ106, a plasmid identical to pIT337 except that the bovine growth hormone gene replaces the IPS gene¹³. In all cases, penicillinase was added to the portions of the reaction mixture placed in the wells at the 3 o'clock and 9 o'clock positions to show that the antimicrobial activity produced was sensitive to inactivation by penicillinase.

Methods. A, Cells were grown in 1% Bacto-tryptone, 0.5% Bacto-yeast extract and 1% NaCl (TY) with (RV308/pIT337) or without (RV308) 100 $\mu\text{g ml}^{-1}$ kanamycin. Cells were grown at 30 °C overnight and then diluted 1:50 into fresh broth and incubated at 37 °C for 6 h. One ml of the culture was then centrifuged, and the cell pellet was resuspended in SDS sample buffer and electrophoresed according to the method of Laemmli¹⁸. B, One ml of *E. coli* cells grown as described for A were pelleted by centrifugation and washed in 10 ml NaCl. Pellets were resuspended in 0.05 M Tris-HCl pH 8, 0.01 M KCl, 0.01 M MgSO₄. Cells were disrupted using a Sonifier Cell Disruptor with microtip, at its recommended maximum power: six series of 5-s bursts with 60-s intervals were conducted. Debris was centrifuged out and aliquots (25, 50, 100 and 150 μl) were assayed for isopenicillin N synthetase activity according to Shen *et al.*¹⁹.

kanamycin phosphotransferase, which is also encoded on the *E. coli* expression vector.

Crude cell-free extracts from cells containing pIT337 also contain IPS activity. Extracts were incubated with LLD-ACV and the reaction mixture tested for antimicrobial activity on agar plates impregnated with *Micrococcus luteus*. As shown in Fig. 4, generation of antimicrobial activity in the assay was dependent on the presence of the IPS gene in the *E. coli* expression vector and on exogenous addition of LLD-ACV. These results indicate that the open reading frame from pIT335 encodes a polypeptide with IPS activity, and that the polypeptide is expressed in an active form in *E. coli*.

The cloning and expression of the IPS gene from *C. acremonium* will facilitate further analysis of the penicillin-cephalosporin biosynthetic pathway. The enzymatic properties and structure/function relationships of IPS can now be tested

in more detail because of the greater availability of the synthetase. The properties of the *Cephalosporium* gene, especially with respect to the control of transcription, can also be studied. Control of expression of the IPS gene is particularly interesting because β -lactam antibiotics are usually produced late in the growth cycle, suggesting the possibility of control of expression of the biosynthetic genes themselves. These aspects are presently under investigation.

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Impaled on Morton's fork

British archaeologists, bent on organizing a successful international conference next year, should have found a more honourable way out of their problem over South African apartheid.

By the time this issue of *Nature* reaches its readers, the British National Committee of the International Union of Prehistoric and Protohistoric Sciences (IUPPS) will have made the most difficult decision ever to have come its way. For the best part of a year, the committee has been planning the 11th World Archaeological Congress, arranged for Southampton next September (see *Nature* 31 October, p.754). Just two months ago the organizers found themselves in what they judged to be an impossible position. Local interests at Southampton had complained that they would boycott or even disrupt the congress if scientists from South Africa were allowed to attend. The committee seems to have spent the summer agonizing about the problem, and the obvious conflict there would be between a decision to give in to opponents of the conference and the ringing declaration of the parent body, IUPPS, there should be no discrimination in its proceedings on the grounds of "nationality, philosophical conviction or religious faith". The issue was further complicated by the circumstance that the chairman of the British organizing committee, Professor John Evans, is also president of IUPPS. But in the end, the organizers decided that they had better bow to pressure to disinvite South Africans. A note to that effect was included in the final version of the conference programme.

This week's meeting (on 20 November) has the unavoidable purpose of mulling over the damage that has been done to international scholarship, to the reputation of British academic institutions and, more practically, to the chances that the planned congress will be a success, financially as well as intellectually. There are three choices: to stand by the decisions already made, to go back on them (letting the opposition go hang) or to cancel the congress (perhaps in the hope that it might be possible to hold it elsewhere at some other time). It probably matters very little what the committee decides. Most of the damage has already been done.

The rights and wrongs of the underlying issue are widely understood. The South African system of apartheid, one of the few systems of racial discrimination enshrined in a nation's constitution, is wicked, and should be done away with. Most people agree with that. In the past few years, external pressure has helped to moderate the South African government's policies, and there is now a chance that further pressure will make the system of apartheid untenable. That proposition (or package of propositions) is less unanimously accepted. Plain speaking certainly helps. Economic sanctions have dubious direct effect, but may have symbolic value. The steps taken by the governments of the British Commonwealth under the Gleneagles agreement to make it difficult for sportsmen who play games in South Africa then to play the same games for their own representative teams have been an endless source of trouble but are probably nevertheless worthwhile, given South Africa's fondness for most kinds of games. But the pressures to which the organizers of the Southampton congress have been subjected appear to derive from the belief that if South African scientists are denied the chance to play a part in international conferences, their government will similarly cave in. To say the least of it, that proposition is untested.

Whatever the chances, there seems no doubt that IUPPS and its British organizing committee should have resisted the pressures

to which it was itself exposed during the summer. IUPPS is not, after all, the government of South Africa. Its constitution does include the stirring declaration that its proceedings are open to all. The British committee's two public statements of its reasons for disinviting South Africans, on 19 September and 22 October, refer to the decision as "hard" and as "taken under duress"; the second "keenly regrets the breach of the principle of free academic interchange". The first refers to the "pressure from several official and worthy organizations" in Britain "which maintain a policy of a total academic boycott of South Africa". The second more explicitly explains that if some of the proposed sponsors of next year's congress, the Southampton City Council in particular, were to withdraw support, the organizing committee might lose as much as £100,000, a quarter of the congress budget. Plainly one reason why the decision was so hard to make was that the committee members were forced to swallow their pride for lack of financial independence.

Nobody should take advantage of the committee's plight to throw brickbats at it. There is no reason to suppose that its members share the views of the pressure groups complaining that South Africans might attend the congress, and which have threatened trouble if they do. But it is fair to ask why the committee, conscious as it appears to have been of the principles of academic freedom, should have caved in so quickly and privately. At the least, it might have made a public issue of its dilemma, pleading the aid of other academic organizations or of academic opinion in general. The committee may have thought it had a duty, in the summer, to act quickly so as to make its conference a success, but by what right did it assume the responsibility of electing itself to be the first academic body to promulgate discriminatory regulations against fellow academics?

For the academic community as a whole, the objective now should be to forfend against future trouble of the kind that has afflicted the British offshoot of IUPPS. The Southampton City Council, the largest single sponsor of the planned conference, seem to have influentially twisted the organizers' arms: the lesson is that academics should not accept support if there are, or may be, strings attached. Student organizations seems to have been influential at Southampton in threatening to deny accommodation to visitors if South African scholars were among them, but it is far from clear how students to whom universities rent accommodation during term-time can decide what happens to it during the vacations, when they pay no rent. But particular responsibility for the IUPPS dilemma, and perhaps even for the collapse of a worthwhile conference, attaches to the Association of University Teachers, which has pursued a policy of "academic boycott" in relation to South Africa; is that what British academics wish their representative body to be saying?

To resist these pressures would of course be inconvenient. Congresses would be less grand if city councils were less generous, and would be less well attended if there were not cheapish student accommodation in which people could stay. There is also a danger that scientists from other countries would stay away if South Africans were free to attend, impeding the process of scientific communication. But scientific organizations are not powerless in the face of such difficulties. Plain speaking, and persuasion in the interests of tolerance, are freely at their disposal. They are tools too little used. □

US science budget

Congress sets upper limits for federal agencies

Washington

THE US Congress has not done as badly by research this year as many had feared it would. In spite of the strong political pressures to be seen doing something to reduce the federal budget deficit, attempts to freeze some appropriations at 1985 levels were defeated. But Congress conspicuously failed to meet the administration's budget requests for several agencies in the new financial year. The National Science Foundation (NSF) and the National Aeronautics and Space Administration (NASA) are among the casualties.

NASA's budget for research and development in the year which began on 1 October is fixed at \$2,757 million, the figure that had been urged by the House of Representatives; the Senate had wanted an extra \$20 million. The sum now agreed represents an increase of \$335 million over last year's figure, but is significantly less than the \$2,881 million the administration had requested.

Specifically, Congress has denied the administration's request for \$226 million for development of the space station, putting a cap on the level of spending next year at \$205 million. Of this, \$5 million is set aside for the robotics and automation thought necessary for the initial operating capability of the space station. Congress seems concerned that budgetary restrictions in future might hold up space science if the administration persists in its plans for a permanently manned structure from the start.

A cap of \$5 million (with a further \$5 million available upon request) has also been put on development of the solar optical telescope, a 1.25-metre instrument that would form the basis of a solar observatory on the space station. This will mean a significant delay for the project, for which \$30 million had been sought in 1986. Other capped projects include: upper stages (\$122 million); the space telescope (\$127 million); the gamma-ray observatory (\$87 million) and the Galileo spacecraft (\$40 million). The funds allotted to space flight, control and data communications are \$3,398 million, about \$100 million less than requested.

The total appropriated for research and related activities by NSF is \$1,352 million, an increase of about \$40 million over last year. The administration's request had been for \$1,398 million. The conference report of the appropriation bill dealing with NSF (in which differences between the House and Senate are patched up) specifically notes that sufficient resources must be made available to support advanced scientific computing and the ocean

drilling programme, as well as efforts to increase women's and minority participation in science and engineering.

NSF is also given \$115 million for the US Antarctic programme and \$55.5 million for science education, which represents a compromise between the figures urged by the Senate and the House. The foundation also has (to Congress's displeasure) \$31.5 million in its science education budget unspent from last year, so that the total for this purpose will be \$87 million, a dramatic increase on what was spent last year. The Very Long Baseline Array is, however, capped at \$9 million;

astronomers had hoped for \$11.5 million for the project. The increase in NSF's total budget, at close to 2 per cent, is in striking contrast to the 15 per cent increase it got the year before.

Congress has also, for the first time in several years, passed an authorization bill for NSF. The authorization may have already been influential in persuading Erich Bloch, NSF's director, to restore a \$1 million programme on ethics and values in science and technology which he had previously planned to axe. Otherwise, the bill will have little immediate effect, although it is likely to focus attention on particular areas such as fundamental engineering research and research facilities in universities, which NSF is now directed to study systematically. The bill will also give the National Science Board the authority to delegate grant approval authority to the director.

Tim Beardsley

British research funds

Government helps to plug dyke

BURIED in the British government's disappointing announcement last week of its spending plans for higher education and research in 1986-87 is some good news for the research councils, which will have an extra £15 million a year to spend. This is seen to result partly from a sombre report on the loss of British scientists overseas prepared by the chairman of the Advisory Board for the Research Councils (ABRC), Sir David Phillips, and published last week.

The Chancellor of the Exchequer's autumn budget statement last week makes it plain that the recurrent budget of the universities is to be held at the levels proposed in January this year, which have been estimated to entail a cut of 1.6 per cent in real terms. Specifically, the university budget will be 2.5 per cent greater in cash terms next year than this, a rate of increase less than the inflation rate.

The research councils are dealt with more generously. The budget statement says that there will be an extra £15 million a year for each of the next three years, in addition to last year's emergency grant of £27 million (spread over three years). A further £10 million will be distributed to selected university research centres for the purchase of equipment.

The immediate explanation of the government's generosity seems to be that Sir Keith Joseph, Secretary of State for Education and Science, has been persuaded of the reality of the rate at which technically qualified people are being lost to Britain. ABRC has been warning the government regularly about the numbers of scientists moving overseas, but the government has repeatedly dismissed the claim, made most forcefully in ABRC's *Science and public expenditure* statement earlier this year, on the grounds that the

evidence is "anecdotal".

ABRC has therefore carried out a survey among 40 leading research groups in Britain (38 in universities and two at Medical Research Council (MRC) units). It now says that its data reinforce its "firm impression" that there is a serious loss of talent, not only by emigration elsewhere but also to industry and to careers in Britain not based on science.

According to ABRC, the reasons given for the loss of talent are "remarkably consistent": more opportunities elsewhere, better pay and prospects, better facilities, frustration about difficulties in winning research grants in Britain, aggressive recruiting by overseas employers and the greater receptiveness of US industry to new ideas.

Although none of these is new, scientists agree that the gap between the United States and Britain has widened in the past few years. Dr Sydney Brenner, director of the MRC Molecular Biology Laboratory in Cambridge, told ABRC that British university research (with a few exceptions) has not been able to keep pace with international standards and has often declined to a point from which recovery is impossible. Engineering was singled out by ABRC as a discipline in urgent need of support; the two Cambridge engineering departments included in the survey reported that 75 per cent of their recent research students have been lost to overseas appointments.

ABRC has not yet decided how to divide Sir Keith's extra money among the four research councils. But as long as the government continues its last-ditch approach to science funding, the brightest scientists are unlikely to prefer an uncertain home career to the tangible rewards elsewhere.

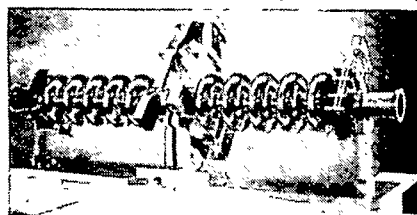
Maxine Clarke

US accelerators

Virginia sets its sights higher

Newport News, Virginia

Less than a year after being appointed director of the Continuous Electron Beam Accelerator Facility (CEBAF), Hermann Grunder is proposing a radical change of design for the accelerator to be built here on a green-field site starting in 1989 — if Congress approves. Grunder wants to abandon the previously-canvassed combination of room temperature linear accelerator and pulse-stretching storage ring and to use instead superconducting



Cornell-CEBAF superconducting niobium electron accelerator element. Each element includes five radio frequency cavities.

niobium radio-frequency cavities to build a continuous-wave electron accelerator, which is claimed to give superior performance for the same cost.

Part of the interest of the project is political; two years ago, the Southern Universities Research Association stole the project from under the nose of the Argonne National Laboratory. Technically, its importance is that it will be the only source of virtually continuous electron beams in the GeV range.

The proposal is being presented this week to the Department of Energy, which will decide by 1 December which design to support. The new design would consist of two antiparallel 0.5 GeV linacs connected end to end by arcs; electrons would circulate around the ring 4 times for a maximum design energy of 4 GeV. That the accelerator should produce a beam that is almost continuous in time is central to its purpose, as such a beam allows "coincidence" measurements to be made; the new design produces a continuous beam directly rather than relying on a pulse-stretching ring.

The advantages of the new design compared with the old are that the energy spread of the electrons will be reduced by a factor of 10; emittance (a measure of beam size) will be reduced by a factor of 100; and power consumption will be reduced by a factor of three, to 6 MW, because of the much lower energy losses in superconducting magnets. Furthermore, electrons will at any time be available at four different energies, corresponding to the number of revolutions completed.

Grunder was recruited to CEBAF from Lawrence Berkeley Laboratory at the beginning of this year, when enthusiasm for the project was waning. The idea of a high-duty cycle accelerator working in the 0.5–4 GeV energy range has been talked

about in the nuclear physics community since 1976, but it was not until 1983 that the CEBAF proposal submitted by the Southern Universities Research Association was recommended to the government for development funding, after a bitterly contested fight with Argonne National Laboratory. Grunder was tempted to the post of director with assurances that substantial research funds would be available in 1987 and 1988 so that he could conduct a complete technology review.

Grunder describes the superconducting continuous wave design as a "responsible risk" proposal. The concept was first demonstrated in the 1960s; advances in technology since then have made it a desirable alternative that has been investigated at several accelerator research centres and developed most recently at Cornell University. Commercial manufacturers are already able to supply the high-precision high purity niobium cavities used.

The design target adopted is 5 MeV per

metre, but in tests some cavities have already performed well above this level. The cryogenic system, which would operate with low-pressure liquid helium at 2K, is large but well within commercial manufacturers' present-day capabilities, according to Grunder. And the need for very high power klystrons, which were the target of a major development effort for the old design, is avoided altogether. Grunder estimates that the new design can be built for the same or slightly less than the \$236 million price-tag for the previous proposal.

The design energy of 4 GeV will enable quark cluster in the nucleus to be examined, but to look for semi-free quarks many physicists believe higher energies will be necessary. The new design could easily be upgraded later to increase energies two or threefold. Grunder is confident that he will be able to sell his new design to the Department of Energy and to Congress: he would, he says, rather be introducing a new design option even at this late stage than fielding questions about why the superconducting option was not considered.

Tim Beardsley

Embryo research

Chances of legislation fade

MR Enoch Powell MP's Unborn Children (Protection) Bill, already killed twice in the British House of Commons this year, has spluttered into life again, although its chances of prolonged survival are slim. Powell's bill will ban *in vitro* manipulations of human embryos, excepting only cases where a particular pregnancy is to be brought to term. If the bill becomes law, Britain's *in vitro* fertilization programme would halt.

Since the death of the bill, popular with many MPs, by filibuster, Mr Barney Hayhoe, a supporter of the bill, was appointed Minister for Health in the summer cabinet reshuffle, in succession to Mr Kenneth Clarke, an opponent. Hayhoe's personal views, however, are unlikely to lead him to introduce legislation that the Prime Minister, Mrs Margaret Thatcher, is known not to support.

Mrs Thatcher is understood to favour legislation to implement the recommendations of the Warnock Committee, published last year after two years of deliberations. But the government's legislative programme for the coming years, embodied in the Queen's Speech two weeks ago, says nothing about the subject, suggesting that a government bill will not make its appearance for at least a year.

So opponents of embryo research are again left to the device of the Private Member's bill, the draw for which took place last week. Powell himself drew 13th place in the ballot, so will be unable to introduce his bill himself. But several of the 20 names in the ballot are supporters, and one of these, Mr Tom Clarke, drew

first place. But Clarke prefers a less contentious subject that stands more chance of success. Although Powell's bill had a majority of support in the Commons when originally debated, MPs now seem to have moderated their views.

Part of the explanation seems to be the emergence of a group called *Progress*, including physicians and medical organizations who are seeking to educate MPs and encourage a more "rational and humane judgement".

Meanwhile, scientists using *in vitro* fertilization techniques have been registering with the voluntary licensing authority set up by the Medical Research Council and the Royal College of Obstetricians and Gynaecologists earlier this year. Eleven centres have been approved by the authority so far; eight more are likely to apply for a licence soon. And evidence that the authority is not just a public-relations exercise is provided by three centres whose initial applications were turned down, two of which have now modified their work to conform with the guidelines and have been given licences, and the third of which is expected to reapply soon. All the approved projects involve embryos less than nine days old and concern chromosome abnormalities; non-destructive identification of optimum growth media and normal stages of growth; and cryopreservation of pre-embryos. The authority will issue its first annual report in March, when it will give details of the research at the approved centres and identify those that have not applied for a licence.

Maxine Clarke

Japanese honours

Prizes, prizes, prizes . . .

Tokyo

THE first recipient of Japan's new International Prize for Biology is an Englishman: Professor Edred Corner, formerly holder of the chair in tropical botany at the University of Cambridge and now, at almost eighty years of age, in active retirement.

The prize was established this year to celebrate the sixtieth anniversary of the accession of the Emperor, himself a noted amateur marine biologist with his own research laboratories. At ten million yen (US \$50,000) in cash plus a medal and a gift from the Emperor, the prize is meant to rank among the international heavyweights. Indeed, to show its importance the Crown Prince, the Prime Minister and the Minister of Education, Culture and Science all turned out to make speeches at the award ceremony. The Emperor too received Professor Corner (who has met him informally in past years) in formal audience at the Imperial Palace.

NIH veto doomed

Washington

CONGRESS seems likely shortly to overturn a presidential veto of the controversial reauthorization bill for the National Institutes of Health (NIH). The bill, which was vetoed by President Reagan on 8 November, would establish a new National Institute of Arthritis, Musculoskeletal and Skin Disease and a new centre for nursing research. It also lays down guidelines on, among other things, how NIH should use peer review for grant applications and on fetal and animal research.

The House of Representatives has already voted by a large majority to overturn the veto; the Senate seems to be in like mind, but a vote will probably be delayed until President Reagan returns from his summit meeting in Geneva.

The President also vetoed an NIH authorization bill last year, but this year's version has commanded stronger support in Congress. Many of the line-by-line budget recommendations in last year's bill have been dropped. And President Reagan has no objection to the creation of the arthritis institute, having directed NIH to go ahead and create the new institute anyway. It will be formed by partitioning the existing National Institute of Arthritis, Diabetes and Digestive and Kidney Diseases.

But the proposed centre for nursing research and a requirement that NIH spend not more than 5.5 per cent of its income on administrative expenses are regarded by the White House as unacceptable. Nevertheless, NIH officials say, many of the administrative recommendations in the bill are likely to be incorporated into NIH practice even if the presidential veto stands.

Tim Beardsley

It is hoped that the prize will become the "Nobel Prize" of biology. This year, the field chosen for the prize was taxonomy; what it will be next year has yet to be decided. But whatever is chosen, it will be critical to the long-term future of the prize that links are made to a vigorous international network of people willing to recommend candidates.

Professor Corner is the author of several standard works of tropical botany much used by Japanese and other researchers visiting South-East Asia and was responsible for a new classification of the genus *Ficus*; he is also a key figure in the establishment of the modern taxonomical systems for fungi.

Although the International Prize for Biology must rate as Japan's most distinguished award, it is not the only prize being given away in Tokyo this week. Professor Carl Sagan is also receiving ¥10 million as part of the Honda Prize set up by the Honda Motor Company. The prize is for "ecotechnology" and Sagan receives it for his contribution toward "ecotechnological exploration of space, as well as toward establishment of a theory of technological development for the new era of civilization based on a more cosmic viewpoint".

There has been a miniature boom this year in establishing new prizes. In the spring, it was the new "Japan prize" which at ¥50 million rivals the Nobel Prize financially and is intended to reward applications of basic science (see *Nature* 315, 616; 1985). A few weeks ago it was the turn of the Kikawada Foundation. This again went to an Englishman, Professor Christopher Freeman, deputy director of the Science Policy Research Unit at the University of Sussex.

But while Japanese companies are generously giving out prizes to foreigners one after another, they are having less luck in finding an agreeable reason for the failure of Japanese scientists to collect top international prizes. Eight officials of the Nobel Foundation, including board chairman Sune Bergstrom, visited Kyoto last week where they received the special prize of the Kyoto Prize. Speculation that the visit would help more prizes to come to Japan, rather than the United States and Europe, were quickly squashed, however. Committee members pointed out that science is international, and that language and distance from Sweden are no barriers to receipt of a prize. An explanation of why a Japanese scientist has never received the Nobel Prize for Physiology or Medicine is rather to be sought in lack of basic research effort. And that might suggest that the new foundations would have served their country better by making up for the lack of funds available for original research in Japan rather than seeking glamour from abroad.

Alun Anderson

French defence

Smaller warheads planned

FRANCE is to develop new miniaturized nuclear warheads to "punch holes" in other people's defence systems, according to the French Minister of Defence, Paul Quilès. In a rare public account of French nuclear policy, Quilès said last week that he had set the Commissariat à l'Energie Atomique several ambitious targets to be achieved by 1994, when the first of a new series of nuclear submarines will come into service.

The miniature warheads are among these objectives, and seem to be a kind of French response to US plans to develop defences against ballistic missiles, the Strategic Defence Initiative (SDI). The more attention the superpowers pay to strategic defence, Quilès said, the more necessary becomes "the penetration capacity of our missiles . . . to our policy of nuclear dissuasion".

Quilès nevertheless repeated last week his government's opposition to SDI. The period when the Soviet Union and the United States were installing the new technology would be a "particularly critical time" for world security, while SDI would be ineffective against the short and medium range missiles aimed at Europe. But it is plain from what he said that France is most of all concerned that the new interest in strategic defence will permit an improvement of the conventional Soviet anti-ballistic missile system already deployed around Moscow. The use of "stealth" technology, both miniaturization and the use of non-reflecting radar coatings, is a natural countermeasure.

In passing, Quilès has also explained French sensitivity about the present programme of nuclear testing in the Pacific, indirectly responsible for the sinking of the *Greenpeace* ship in New Zealand and, ironically, for the resignation of Quilès's predecessor, Charles Hernu. France is obviously committed to a new programme of warhead technology, for which testing is considered essential. In France, there appears to be no opposition to the programme.

From what Quilès said last week, it also seems that the French navy rather than the air force will be the spearhead of the new strategy. Noting that the mere presence of British nuclear submarines in the South Atlantic had kept the Argentinian navy in port during the Falklands war, he announced that France would add another nuclear submarine to the six ordered since 1984. But Quilès effectively squashed air force hopes to build a new land-based strategic missile, saying that the resources would be better spent on the improvement of submarine missiles. The existing "park" of 18 missiles in Haut-Provence will, however, be retained.

Robert Walgate

Computer language

When to go back to BASICs

Dartmouth, New Hampshire

BASIC is being debased, says one of its creators, Professor John Kemeny of Dartmouth College. Kemeny, still at Dartmouth's department of mathematics, where BASIC was developed more than twenty years ago, is now calling for a halt to the development of inferior versions of his never-patented language, saying that they are corrupting its purposes and causing chaos in the computer industry as well.

In 1963, Kemeny and his colleague Tho-

mas Kurtz changed the face of computer science forever by inventing BASIC, now probably the most widely used computer language in the world. Their objective was to create a novel learning tool for students. To begin with, they installed a collection of teleprinters linked with a mainframe in one part of a students' recreation room.

The arrival of the microprocessor made sure that BASIC would not be just a Dartmouth teaching aid. Now, after twenty years, Kemeny reckons that there are more than 100 versions of his language, most of them so inferior that he and Kurtz have become seriously concerned.

Kemeny says that the later versions violate the philosophy of the original. If only users would go back to BASICs, educationalists would have a superior tool, while programs written for one microprocessor would be found to work on others. BASIC (which stands for Beginners' All-purpose Symbolic Instruction Code) began, he says, as a device for giving undergraduates simultaneous access to Dartmouth's GE mainframe computers. With the volume production of microprocessors in the 1970s, however, the language began to be used extensively in various forms. Because the memory of the early machines was limited, programmers were at first compelled to improvise, simplifying BASIC and specializing it to fit the memory of small machines.

Kemeny's frustration is that, even though the microprocessors now available do not have the limitations of those being manufactured 20 years ago, the omissions

from BASIC used by the early programmers have not been reinstated. But, Kemeny says, "these machines are not toys, but very powerful computers. The IBM PC I have at home is not a particularly expensive model, but it is more powerful than the original timesharing machine here at Dartmouth." That is why, Kemeny says, many of the BASIC programs now on offer "just don't live up to what any computer scientist would expect of a good language in 1985".

Two years ago, Kemeny, Kurtz and three other computer experts accordingly set up a company to promote the fundamentals of BASIC languages, and to engineer improvements of software developed by other computer scientists. Inevitably, the company is called True Basic.

Kemeny predicted 20 years ago, when opening the computer centre at Dartmouth College, that every home would have a computer terminal within a quarter of a century. The unexpected arrival of the microprocessor has almost made that prediction a reality, but the lack of a common computer language is still an impediment. So one of the goals of the Kemeny group is to develop the language that will make the vision a reality.

So far, the group has developed compilers for the IBM PC and for the Apple Macintosh that will allow them to respond to TRUE BASIC. Two new microprocessors to be launched onto the Christmas market this year will also have such compilers. According to Kemeny, because the compilers written for the individual models embody the necessary changes, programs written in TRUE BASIC for any of these models will run successfully on any other. **Bill Johnstone**

India against AIDS

New Delhi

ALARMED by reports of AIDS (acquired immune deficiency syndrome) in Pakistan, both India and Bangladesh are taking precautionary steps to keep out the AIDS virus. The Indian Council of Medical Research (ICMR) has set up a task force headed by the ICMR chief Dr V. Ramalingaswami. A similar panel under Professor Nurul Islam, director of the Dhaka Institute of Postgraduate Medicine, has been set up in Bangladesh.

No confirmed case of AIDS has been reported either in India or in Bangladesh. But Professor A.N. Malavya of the All-India Institute of Medical Science in New Delhi and a member of the task force says it is only a matter of time. The major aim of the task force is to educate both the doctors and the public about AIDS without creating a scare and to keep tabs on vulnerable groups including haemophiliacs, homosexuals and a significantly large population of eunuchs besides the prostitutes in ports such as Bombay, Calcutta, Madras and Vishakapatnam.

Cases showing AIDS symptoms will be referred to any of the dozen medical institutions to be designated as reference centres. The National Institute of Virology in Pune is being equipped to screen for AIDS antibodies and viruses in blood samples of suspects. The task force is evolving a detailed questionnaire to be circulated to private and government doctors and an educational campaign is planned. But the Indian government does not want to divert huge funds to an anti-AIDS programme as there is no evidence of AIDS yet.

In Bangladesh, the AIDS committee has been asked to prepare a plan of action for the prevention of AIDS. As well as evolving a programme for surveillance, detection and treatment, the committee intends to propagate Islamic ideals on the evils of promiscuity. People have stopped buying imported second-hand clothes following a rumour that they might be contaminated with AIDS virus, and Bangladesh newspapers have asked the government to screen incoming passengers from the West.

K.S. Jayaraman

UK laboratory animals

Debate on legislation ahead

THE British government's Animals (Scientific Procedures) Bill, published last week, will be debated in the House of Lords this month. The bill follows the proposals outlined in two white papers (policy documents), Cmnd 8883 in May 1983 and Cmnd 9521 in May 1985, and is intended to replace the Cruelty to Animals Act of 1876 (see *Nature* 315, 267; 1985).

Attempts to reform existing legislation on laboratory animals have failed in the past because of strong protests from animal rights groups and reluctance of members of parliament (MPs) to become embroiled in the issue. The new bill is supported by the middle-of-the-road animal welfare groups, who advised in drawing it up, but is violently opposed by the extreme animal rights groups who are against any experiments on animals being made legal.

The bill requires that a project licence be obtained before work can begin, con-

trols breeders and suppliers of animals and establishes a committee to provide independent advice to the Home Secretary. All research establishments must appoint a named person to care for animals, there will be heavier penalties for offences and controls on the re-use of animals.

A significant provision is the explicit acknowledgement that pain is caused to animals; licences will be issued only if the Home Secretary is satisfied that the benefits outweigh the adverse effects on the animals and the licence requires that pain, distress and discomfort are minimized.

The bill has wide support from scientists and welfare groups, and its passage through the House of Lords seems assured. But the Commons is very different. MPs have traditionally been extremely reluctant to favour any legislation that makes them seem to be supporting "cruelty" to animals in the eyes of their constituents. **Maxine Clarke**

British research councils

Budgets on a shoestring

PROFESSOR Bill Mitchell, new chairman of the largest British research council, the Science and Engineering Research Council (SERC), presented the organization's annual report last week. Sir John Kingman, who was chairman until the end of September, says in the report that the severe financial pressure of recent years has resulted in losses sometimes "out of all proportion to the money saved", particularly in the lack of provision of operating costs for big-money facilities. "The truth of the matter is that the United Kingdom is trying to maintain its science base on the cheap", said Kingman.

Mitchell, taking a more optimistic line than his predecessor, congratulated Kingman on "coping so well in difficult circumstances" but said that the work described in the report was not that of a "demoralised science and engineering community". But like Kingman, he stressed the role of basic research in making discoveries of "economic significance".

One new source of money in the science arena is a joint Ministry of Defence (MoD)/SERC programme. SERC welcomes the influx of money (MoD and SERC will each provide 50 per cent). Projects will be subjected to peer-review in the normal way, and will be restricted to unclassified fields. SERC will assess the scheme next year and "reconsider" if any of its conditions are not met.

The highlight of 1984 was the production of the first neutrons by the Spallation Neutron Source (Rutherford Laboratory, near Oxford) in September. At the Synchrotron Radiation Source (Daresbury Laboratory, near Manchester) the "wiggler" beamline enabled the first enzyme crystal, phosphorylase *b*, to be monitored as it performed its catalytic reaction. Unexpected order-disorder transitions have been found and people are queuing up to use the device which measures 40,000 reflections per second.

In 1984 the Isaac Newton, Jacobus Kapteyn and Carlsberg Meridian Circle telescopes began operation, the Active Magnetron Particle Tracer Explorer satellite was launched and the Anglo-Australian Telescope has allowed the most distant galactic objects yet known to be seen. But the Royal Greenwich Observatory may soon stop participating in the international time determination programme (see *Nature* 31 October, p.754); SERC has decided to support observational astronomy at the expense of other projects.

Engineering science has received increased financial support during the past 10 years so that it now makes up 20 per cent of SERC's budget. SERC is reviewing the effectiveness of its engineering programmes and will decide whether to shift priorities again, or even whether to change its own name (to STRC, T for

technology) in February 1986. Similarly, SERC is reviewing its position on chemistry research, an area that has lost ground in the past three years.

Another controversy caused by government cuts is the question of British membership of the European Organization for Nuclear Research (CERN). Reduced money to SERC has coincided with an increased subscription to CERN and it is likely that some experiments will stop completely, damaging Britain's reputation as a reliable partner in international collaborations. The decision is in the hands of the government, at present considering the recommendations made to it

Soviet plan

More goods, more science

THE "Draft Basic Guidelines" for the Soviet Union's Five-Year Plan (1986-90) are, as usual, not so much a detailed economic programme as a manifesto eulogizing the Soviet way of economic life. The preamble extols the progress in the "scientific and technical revolution" during the past five years. It says that nuclear power now accounts for one tenth of all Soviet generating capacity and that the Soviet Union, "which earlier took first place in the world in terms of oil extraction", now holds a similar position with regard to natural gas. Free textbooks are now provided in schools, the housing problem is "being consistently solved" and the natural wealth of the eastern and northern regions of the Soviet Union are being opened up at an "accelerated rate".

The guidelines for the future present a somewhat less happy picture. The "supreme goal" in the next five years and for the remainder of the century is the "steady upsurge of the standard of living" of the Soviet people by raising the efficiency of production. In pursuit of this aim, the food programme (Brezhnev's last legacy to the Soviet people) is to be implemented to ensure the "scientifically substantiated norms of rational consumption", while there is a package of measures to improve the health service. There have been complaints in the Soviet media about poor performance in the health service, perhaps because the energetic anti-alcohol campaign led to the neglect of other aspects of health.

The emphasis on the citizen-consumer reveals, perhaps, how much this sector has been neglected in previous plans. Production of non-food consumer goods is to be increased by more than 80-90 per cent, and the "volume of services" delivered will, according to the forecasts, be more than doubled. But other targets, too, are set equally high — gas extraction at 60-80 per cent more than in the current quin-

quennium and nuclear power increasing between five and sevenfold. In industry, there is to be a general inventory of "fixed production capital", and more than one third of it renewed. The opening up of Siberia and the far east to industry is to continue, although there is now increased emphasis on environmental protection.

In science, the role of the Soviet Academy of Sciences as co-ordinator of research is to be "enhanced", as also will be the role of the academies of the union republics. The draft plan speaks of utilizing the scientific potential of the higher education system, and expanding the research and development work in the universities. There is to be closer interaction between the research facilities of the academies, the universities and the specialized institutes of the production sector, and "progressive new forms" of research organization will be sought.

Several scientific fields are listed for special development, including theoretical and applied mathematics, information science, cybernetics, particle, nuclear and solid-state physics, micro and quantum electronics and also research into nuclear and non-traditional energy sources. The "scientific principles" of catalysis, chemical technology and biotechnology are to be "elaborated" and the mass production of personal computers is to be "organized".

The supply of modern instruments, materials and other necessities to research facilities will be "substantially improved".

The guidelines are nevertheless unclear about the relative roles of pure and applied science, saying that the "fundamental task" of the current plan is to "strengthen ties between science and production", while, at the same time, "priority significance" is to be given to fundamental research which "predetermines the attainment by social production of a qualitatively higher level".

Vera Rich

by the Kendrew committee (see *Nature* 315, 619; 1985). Money for nuclear physics and astronomy research has generally declined in the past ten years relative to basic science and engineering research. Even so, SERC says it needs an extra £30 million a year by the end of the decade to support a programme of research in the basic sciences "commensurate with the country's standing as an advanced European country . . . and to provide the trained manpower the country needs".

SERC is submitting its first-ever corporate plan to the Advisory Board for the Research Councils (ABRC) this week, and will publish it next month. ABRC will use the corporate plans from all the research councils in its annual advice to the government, in which it makes its case for more money for science. **Maxine Clarke**

Foreigners' tenure at Tsukuba

SIR—I should like to add a gloss to Alun Anderson's fair report on the "turmoil over treatment of foreign staff" (*Nature* 10 October, p.465) at Tsukuba University, self-proclaimed champion of the internationalization of university education in Japan.

Four foreign staff members, including myself, expected to be given long-term contracts not because "some supporters made affirmative responses" to them, as Tsukuba's president, Nobiyuki Fukuda, says, but because the due process for their appointments had been put in train: the vice-president in charge of education, *ex officio* member of the personnel committee that makes final decisions in personnel matters, first ascertained the need for our posts at the departmental level, then discussed his proposal to give us long-term contracts at a meeting of the president and the five vice-presidents, none of whom voiced any opposition. Thereupon he instructed the department heads to let us know that we were scheduled to get tenure. The department heads prepared the formal applications to be submitted to the personnel committee. I therefore think that our expectations were not "extremely strange" as Mr Fukuda believes, but only reasonable. What is strange is that the final authority on personnel matters was not given a chance to make its decision as neither the departmental applications nor the supporting proposition of the vice-president were considered by a meeting of the personnel committee — apparently both were blocked when the vice-president was sacked.

While it is true that there is no final definition of "internationalization", I think there is agreement on certain ingredients that it must contain, such as non-discrimination on grounds of nationality, transparency and minimum civility. Tsukuba University is still in want of all of them, I am afraid. Anderson amply illustrated that foreigners at Tsukuba are not treated "just like anyone else". It might be added that Tsukuba did not avail itself of the opportunity the new law offers to give tenure to foreigners. For foreigners at Tsukuba, "tenure" means just 5-year contracts, while every Japanese staff member gets real tenure from the start. Foreign lecturers have to carry a teaching load of 12 hours per week whereas their Japanese colleagues average five.

The rule limiting employment of foreigners without tenure to four years resulted from the unwillingness of university authorities to speak their mind clearly. Not all foreign lecturers have always performed their task satisfactorily. The university therefore introduced a system grading each foreigner (but no Japanese) each year as A, B or C. Yet rather than tell the Cs to improve or leave, all lecturers had to quit, the As along with the Cs. The

announcement was made in writing, with no explanation.

"Inexplicable silence followed by brazen behaviour", a Japanese attitude regarded by many Westerners and Japanese as a serious obstacle to internationalization, also characterized the recent events at Tsukuba that Anderson described. No wonder then that Tsukuba is seen as becoming more parochial rather than internationalized. To dismiss this view as "nonsense", as Mr Fukuda does, does little to solve the problem.

MARGARET SAWADA

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Reputable PhDs

SIR—"A PhD is supposed to be a training in research" says Beverly Halstead (*Nature* 316, 760; 1985) and therein lies his problem. I suspect that some proportion of every thesis (for example the research training part) will not meet the standards of the existing avenues for publication. That is not to say that some of this proportion would not be useful to someone embarking in that particular area of research. Thus there exists a case for making theses, in their entirety, more readily available globally through some other means (for example microfiche).

Attempting to distinguish major from non-major contributions to science would be headache enough, but differentiating between reputable and non-reputable journals is a job I leave for Beverly Halstead. I look forward to his published list appearing in these columns.

JONATHAN A. KEOGH

Department of Botany,
University of Botany,
University of Otago,
Dunedin
New Zealand

Units are for people

SIR—Although no system is proof against mistakes, spectacular events, such as the apparent misprogramming of a space-shuttle computer (*Nature* 315, 702; 1985), highlight the usefulness of an agreed universal system of units. D.C. Jolly (*Nature* 316, 480; 1985) enters an impassioned case for the foot, pound, second system, as an alternative to SI and pleads for a counter-revolution. W.G. Rees (*Nature* 317, 10; 1985) has suggested an ingenious system which differs from the foot, pound, second system by a possibly tolerable amount, such that c , G and $4\hbar$ become exact multiples of his base units.

Unfortunately, his suggestion is impractical since, for example, we cannot yet measure all of the fundamental constants and atomic phenomena with the required accuracy. Indeed, as we do so, the SI units

too will incorporate the fact, for the Conférences Générales des Poids et Mesures will revise the recommended definitions of the base units accordingly, as they have with the second and metre — but without altering their size.

While it is tempting to argue for a change to a "natural" system of units, we ought to recall that part of the appeal of the c.g.s. system to our nineteenth century forebears stemmed from its use of "natural" quantities associated with our planet. Who can be sure that the fundamental constants e, c, h , G and so on will not have been replaced by an ever deeper set by the end of the next century? Moreover, a system only appears natural if the units are appropriate to the discipline with which the proposer is most familiar — what is "natural" for the physicist is not necessarily so for the biologist, chemist, artisan or "man in the street".

We might yet, if we chose, be able to recycle some of our earlier units instead, for G is near enough unity in the Gunter link, troy grain and lunar month system of units, and the Planck constant essentially 1 attoleague¹ megascruple² centiweek³ (British nautical leagues, apothecaries scruples and sidereal weeks). Professor Kenneth Meyer, at the University of Cincinnati, has shown how unit conversion tables (in the *Handbook of Chemistry and Physics* and elsewhere) together with a microcomputer allow such combinations to be found — even an exact one by an undetectable massaging of the magnitude of the earlier unit! I suggest though that readers wait for the forthcoming CODATA evaluation of the recommended "best values" of the fundamental physical constants before searching for the best such combination of earlier units to six decimal places!

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Sir Henry Wellcome

SIR—In the article on the Wellcome Trust and Foundation (*Nature* 24 October, p.662), you refer to them as being set up under the will of Sir Henry Dale. I believe that it was Sir Henry Wellcome who in his will created these institutions.

As a young prescriptions clerk in Rochester, Minnesota, Wellcome was encouraged and supported by Dr W.W. Mayo to improve his education and not to be satisfied with his position. Mayo had been taught chemistry by John Dalton before emigrating to the United States. His sons, William and Charles Mayo, created the Mayo Clinic.

LARS BRUMER

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Evolution and education

SIR—Your erroneously titled "Californian setback for evolutionists" (T. Beardsley, *Nature* 5 September, p.3) described an unprecedented recommendation by California's Curriculum Commission: that no science textbooks be adopted for grades 7 and 8 unless the books be revised to give proper treatment to evolution and human reproduction, among other subjects. This was a significant defeat for creationists and others who try to subvert science education in this state. The defeat was certified on 13 September, when the Board of Education accepted unanimously the Curriculum Commission's plan.

Beardsley erred when he attributed to California an "antidogmatism law". No such law exists. But the Board of Education has an antidogmatism policy, which dictates: "That, on the subject of discussing origins of life and Earth in public schools: (1) Dogmatism be changed to conditional statements where speculation is offered as explanation for origins. (2) Science should emphasize 'how' and not 'ultimate cause' for origins."

This grotesque declaration, adopted in 1972, is noteworthy for many reasons. First, it is blatantly dogmatic. Second, it can be explained only as a device for appeasing creationists and for abetting their attempts to distort the teaching of science. Third, it erects a wholly specious, intellectually indefensible demarcation between discussions of "origins of life and Earth" and discussions of all the other subjects that science addresses. Fourth, nobody can say precisely what the policy means or requires, because none of its terms — dogmatism or conditional statements or speculation, for example — is defined. Fifth, in an antic, confused decision rendered in 1981, a judge of California's Superior Court suggested that the policy should apply not only to discussions of "origins of life and Earth" but also to discussions of organic evolution. Sixth, since 1981, creationists in California have relentlessly and conspicuously invoked the policy (and their own interpretations of its surrealistic language) during efforts to vitiate or suppress discussions of evolution in textbooks and in science classes. Seventh, because the policy's only evident purpose is to serve the adherents of a particular religious dogma, the policy's constitutionality is (to say the least) questionable.

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Tim Beardsley writes: In the 1981 case referred to by Bennetta, Sacramento Superior court Judge Perluss not only upheld the validity of the antidogmatism policy but also ordered the State Board of Education to disseminate it to all interested parties; the policy may therefore be not entirely without legal force. □

Teenage SCOPE

SIR—Your article entitled "What to make of nuclear winter" (*Nature* 19 September, p.189, mentions "the working party called the Scientific Committee on Problems of the environment (SCOPE) set up three years ago by the International Council of Scientific Unions (ICSU)". This sentence give a wrong picture of our committee. As you might know, SCOPE is one of the ten scientific committees of ICSU and was established in 1969 to assemble, review and assess the information available on man-made environmental changes and the effects of these changes on man.

In 1982, the SCOPE General Assembly decided, at the request of ICSU, to appraise the state of knowledge of the possible global environmental impacts of nuclear war. That project, called ENUWAR, may have been the most important of some ten international scientific projects undertaken by SCOPE during the past triennium, but it does not overshadow the contribution of SCOPE during the past fifteen years to the advancement of scientific knowledge on many problems of global environmental concern.

VÉRONIQUE PLOCO
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Velikovsky's evidence?

SIR—Lynn Rose's defence of his interpretation of the Venus Tablets¹ may be seductive, but his conclusion is neither as inevitable nor as secure as he argues. He states that the tablets "strongly support Velikovsky's theory that Earth has undergone catastrophic orbital change within historic times". However, there is no explicit connection between the tablets and Velikovsky's catastrophes. Indeed, the change implied in the shape of Earth's orbit is a far cry from the intersecting orbits discussed by Velikovsky. The only philosophically justifiable conclusion is that Rose's interpretation of the tablets "is consistent with" Velikovsky's theory, not "strongly supportive".

In contrast to Rose's insistence on ignoring as few of the observations in the tablets as absolutely necessary, he argues his case favouring Velikovsky while ignoring a plethora of contradictory data. The falsification of Velikovsky's scenario provided by Greenland's Dye 3 ice core² is corroborated by ocean sediments and bristlecone pine rings³ and the revised late-glacial Swedish varve chronology⁴. Either their mere existence contradicts Velikovsky or they do not contain debris suggesting a catastrophe. Velikovskian catastrophes are neither indicated by nor necessary to explain the natural stratigraphies of the Holocene.

Furthermore, according to Rose's own

work with Vaughan⁵, the sequence of orbital changes implied by *Worlds in Collision* that conserves angular momentum without increasing orbital energy starts with the untenable condition of Earth closer to the Sun than Venus now is. Surely such a severe physical constraint⁶ is to be accorded more weight in assessing the validity of Velikovsky's theory than an idiosyncratic interpretation of the enigmatic Venus Tablets.

The Terminal Cretaceous Event 65 million years ago, whatever it was, left unambiguous, worldwide signatures of iridium⁷ and soot⁸. The catastrophe Velikovsky conjectured within the past 3,500 years left no similar signatures. In this light, advocating the validity of Velikovsky's scenario, as Rose does, strains the bounds of credulity, both scientific and philosophical.

If the Venus Tablets preserve authentic observations, however disguised by copying errors, then explaining the discordant observations needs to be accomplished with new insight. For example, John D. Weir has suggested to me that if a formerly very bright comet periodically altered the sky's brightness, then the dates of appearances and disappearances for Venus would be affected. The debris from the disintegration of comet Encke 4,700 years ago, discussed by Clube and Napier⁹, would be a possible agent. The clay of the Venus Tablets is far too fragile a foundation upon which to justify a new physics for the rationalization of Velikovsky's theory.

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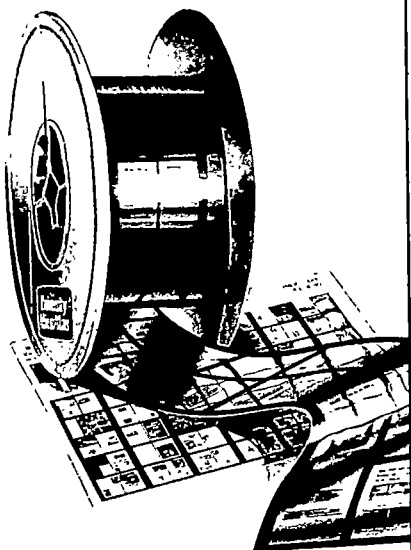
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2. Ellenberger, C. L. *Nature* 316, 386 (1985).
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7. Alvarez, L. W. *et al. Science* 208, 1095-1108 (1980).
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9. Clube, V. & Napier, B. *The Cosmic Serpent*, 150-153 (New York & London, 1982).

Help wanted

SIR—In collaboration with Professor Richard Gregory, Professor Dorothy Hodgkin, Sir Fred Hoyle, Sir Peter Medawar and Dr Jonathan Miller, I am preparing an anthology of science writing, to be published by Basil Blackwell. The intention is to bring together a wide variety of pieces, ancient and modern, of varying length, and drawn from all sectors of science, the sole criterion being that of literary excellence. We would be delighted to receive suggestions from readers of *Nature* regarding items suitable for inclusion in what we hope will prove to be a work of sheer aesthetic and intellectual delight.

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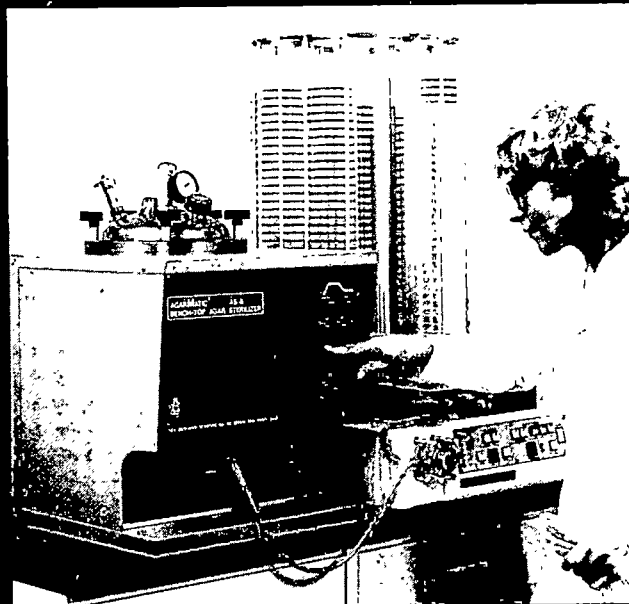
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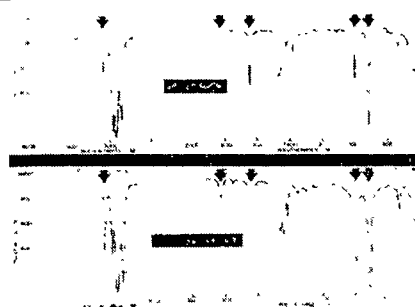
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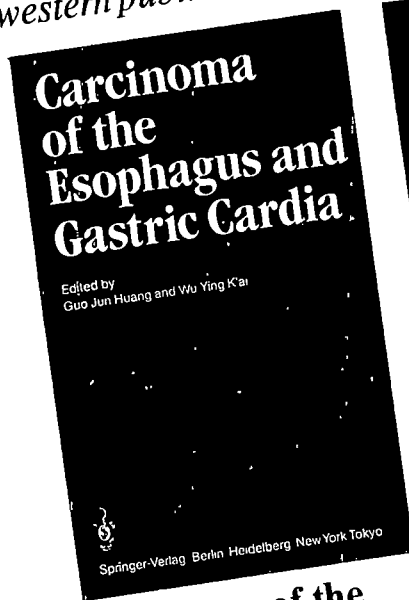


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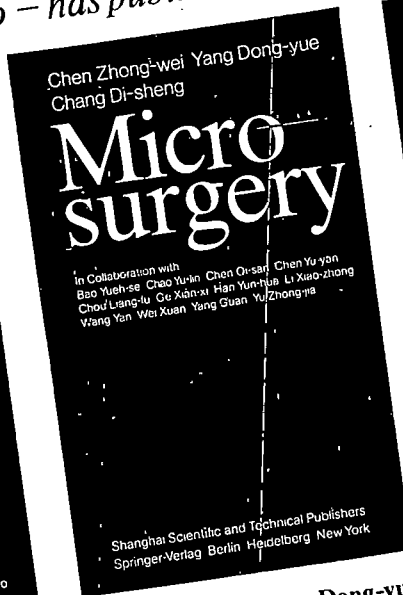
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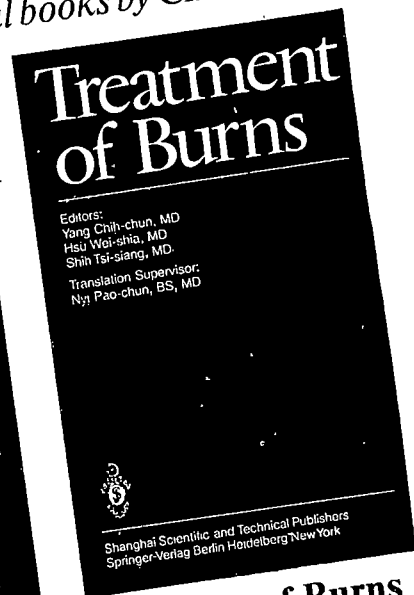
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Reader Service No.6

Industrial power by research?

China's science is in the process of being harnessed to ambitious social and economic goals. This survey of science in China may suggest whether the venture will succeed.

Will China make it? And how soon? Or will the present still-short period in China's recent history prove to be another aberration, like the Great Leap Forward (1960–66) or the Cultural Revolution (1966–76)?

In China now, opinion is unanimous. The largest nation on the Earth is at last on the road to becoming an industrial power matching in prosperity and creativity the most successful nations elsewhere in the world. The job will be done by adapting science and technology to China's distinctive talents, of which the Chinese are self-conscious and proud. The task, of course, will take time — half a century or even more — but what is that by the yardstick of China's three millennia of recorded history?

The optimism is almost palpable — and is the only credible explanation of people's apparent willingness to endure a degree of personal deprivation, material and otherwise, that still shocks people from the West.

This survey of science in China is inevitably incomplete, less a survey in the strict sense than a reconnaissance. The government of China claims that there are more than 4,000 independent research organizations in China, which, if true (there are problems of definition), implies that the account that follows is based on a one per cent sample which is, moreover, biased towards institutions whose reputations travel.

Even so, there are good reasons to believe that important generalizations can be safely made. First, there is the curious phenomenon, entirely unfamiliar in the West, that government officials are the first to agree that there are obvious shortcomings in their organization for China's objectives; often, they will go on to explain what they plan to do about these problems. Second, there is the uniformity of the common conviction that the ship is sailing in the right direction: it would be churlish to ask whether China has always been so certain of itself.

China is also a Marxist state, in this as in many other respects the largest anywhere. Those who may think that the political system must surely be irrelevant to a description of what laboratories are like and to an appraisal of how the system is orga-

nized are profoundly mistaken. The truth, in China, is that communist principles and organizations influence everything, not only the distribution of power between the Party and the government but the conditions under which ordinary people live and researchers go about laboratory work. China's goal is not merely to become an industrial power of the first rank, but to remain a communist state.

For the time being, the disciplines and controls stemming from the party's influence, not to mention the way in which the patriotism engendered by the present optimism is strengthened by the party's control of press and television, may help to keep China moving forward single-mindedly. It is an absorbing but unanswered question whether, somewhere along the road, intolerable contradictions will arise. Meanwhile, it is best to remember that the break with the Soviet Union in the late 1950s came about because the then Chinese leadership believed itself alone to be following the true precepts of Marx.

Enthusiasm

Much of the enthusiasm of the present nevertheless springs directly from the way in which the system that gave rise to the Cultural Revolution is now being changed; "reform" is the slogan. The reopening of China to the outside world is one sign of the change; China has gained a torrent of tourists, and is now looking for the generous transfer of technology from the West. The decentralization of the power to transfer administrative (but not political) decisions to provincial governments and operating agencies of the government is another; efficiency is the goal. An uncovenanted side-effect may be that able people no longer feel that only the local general secretary of the party has real power.

The most daring of the reforms so far has been the much-publicized decision that peasants may sell their produce on free markets wherever they have access to them. One result has been a sharp increase of agricultural production (to which other influences have contributed), another is the persisting argument between the party purists and pragmatists about the propriety (and wisdom) of letting loose the genie of the search for gain.

Yet for now, the economic consequences of reform are its sufficient justification. During the past five years, agricultural and industrial production together have increased at a rate of more than 10 per cent a year, too fast for comfort. So the new five-year plan, presented in outline to a National Party Conference in September, will be based on the assumption that growth in future will be moderated to between 7 and 8 per cent a year, just about enough to meet the objective of the Party Congress in 1982 that there should be a fourfold increase of production by the end of the century.

Enterprise

The temptation to attribute these changes to the mild breath of free enterprise in the economy is strong, but would be mistaken. Obviously, economic motives have helped, but only marginally. Eighty per cent of the population of China still lives on the land, and only a tiny fraction of these people, those who by chance live near cities allowing the import of produce other than through the state purchasing agencies, are able to benefit.

The countryside has become more productive for two other reasons, the improvement of crop strains in the past few years (and their general and rapid adoption under the party's influ-

Explanation of a map

To many in China, the choice of an eighteenth-century map, however interesting, to decorate the cover of this special issue of *Nature*, will seem inappropriate. Why not a rocket taking off, or a power station, or a textile mill or something else that will represent the modernity of emerging China? The choice, deliberate as it is, is intended to be symbolic in several different ways.

First, everybody visiting China must be aware of China's sense of its own antiquity, from which may derive its people's confidence, fatalism and patience; this map puts China at the centre and the rest of the world around the edges.

Second, the map shows China as a kind of island which, in spite of the opening to the outside world in the past eight years, China still is. The changes of the past few years have been dramatic, but China is so huge that its contacts with the outside world remain geographically peripheral; the special economic zones are the modern equivalents of the coastal concessions of the eighteenth and nineteenth centuries. In the cities, foreigners are lavished with hospitality but are otherwise still mostly isolated in the foreigners' hotels from which occasionally they venture to spend foreign currency in the shops set aside for foreigners. □

ence), and the concomitant rapid growth of rural industries (which is China's intended but still untested defence against the usual flight from rural to urban slums). In other words, the science of plant-breeding has already contributed substantially to prosperity.

The same is true in manufacturing industry, but to an unquantifiable extent. Indigenous capital investment in new factories has certainly played an important part. So too have the countless joint ventures with overseas high-technology companies which, by creating enterprises for the indigenous manufacture of modern equipment such as telephones, allow the value of China's output to be increased without a proportional investment of indigenous skill. (McDonnell Douglas, for example, will soon start shipping to Shanghai kits from which civil aircraft will be assembled.) Vast as China is, it seems generally and realistically to be accepted that it will be a long time before the country's technical community can sustain the government's industrial ambitions.

Impediments

Several obvious impediments are listed in what follows, but in a spirit that needs careful definition. China, by its own assessment, is a developing country that has somehow survived two centuries of foreign occupation and civil war to enjoy less than a decade of single-minded social and economic progress. The most remarkable feature of the present state of affairs is that China's ambitions cannot be laughed out of court. Another is that the government, whatever its political character, appears to have a realistic appreciation of what the difficulties will be.

The most serious obstacle to continuing change is the shortage of technical skill. In university departments and research laboratories, the lack of technicians means that creative people spend much of their time on laboratory chores. Nowhere are there the small armies of people needed to make headway quickly in fields where numbers matter — writing software for digital telephone switching systems, for example.

Yet it is only within the past few years that, on the academic side, China has had the confidence to mount its own PhD programmes in universities and research institutes. In applied research, the most glaring shortage is of people competent to mount research and development programmes within the framework of industrial enterprises. The most acute shortages appear to crop up in the medium-sized enterprises which, elsewhere, are the chief agents of technical change.

The government acknowledges the difficulty. The next five-year plan will see an increase of recruitment to the universities (by a modest 20 per cent to an annual intake of 500,000 students), but the chief emphasis will be on the training of technicians, in formal technical colleges and in vocational schools linked with the factories. If there is to be an expansion of the university system that would give China a participation rate comparable with that of say Britain (let alone the United States or Japan), that will be postponed to a later plan.

Often, it emerges in conversation (see p.219, Beijing polytechnic) that there are influential people who think China does not need to follow the models established elsewhere; if the enterprise is properly planned (as communist ideology says it should be), a single research institute can take responsibility for the development carried out in the West by several competing enterprises, so that technical skill will be used more efficiently. There are the makings of future rows in this difference of opinion, but not yet.

Difficulties

A much more serious difficulty is the way the research enterprise is organized. China does not, at present, benefit as it might from the skills at its disposal. China's public institutions and enterprises — until recently, there were no private enterprises — are organized along the lines now familiar in the Soviet Union. At the very top, government ministries more often correspond to what would be called executive agencies in the West (which explains why, in China as in the Soviet Union,

there are so many of them). Their job is not so much to make policy that others follow, but to ensure that predefined tasks are executed. So a hypothetical ministry of electric power will not merely build and operate electricity generating stations but also build factories in which to manufacture generator sets and the other equipment needed. To ensure a supply of trained people, it will found a university or college at which promising young people can be trained, and it will then undertake to provide those whom it employs with housing and their children with schooling. A ministry or other large public organization is like a state within a state, commanding the undivided loyalty of its employees, but bound to them by the duty to provide the necessities of life.

Revolutions against previous feudal systems have plainly been less radical than their sponsors have imagined. In the Chinese Academy of Sciences and its institutes and at other academic institutions, the paternalism of the institution towards those whom it employs is taken for granted by both parties. Specifically, those graduating from universities will be assigned to jobs in commerce or industry within quotas provided by the government, but may also apply for posts at research institutes. If successful, the corresponding quota slot remains unfilled, and the student stands on the lowest rung of the research ladder which may, with further success, offer the chance of a PhD course, perhaps overseas, or even a fellowship somewhere else. Promotion within the institute is the rule; sometimes, a transfer to an administrative post with the parent organization is possible. Salaries are poor but costs are low; people pay a few yuan a month (\$1=2 yuan) for the apartments with which they are provided. At suburban research institutes, there will be communal facilities such as a pond stocked with carp. The University of Beijing runs no fewer than three schools for the education of the children of the faculty.

Benefits

The benefits of such arrangements are plain; young people joining a research institute know that they must expect to succeed, at least in the first instance, within that framework. They are accordingly loyal, diligent and respectful. But if, with the passage of time, they should be less than wholly successful, there is no incentive for them to move elsewhere, and every incentive for them to stay. Research institutes become families, greatly extended families. The managers of the system now recognize that the consequence is often stultifying immobility, and several attempts to tackle the difficulty are under way — the secondment of people to factories without loss of academic status, limited postdoctoral fellowships and the like. But the effects of these innovations will be only marginal while the economic system depends as much on rewards in kind.

The doctrine of the self-sufficient public agency is another impediment to the effectiveness of the professional research cadres. Even if it had been possible to buy liquid nitrogen and

Chinese names confuse

THE romanization of Chinese characters is a problem and in an effort at least to standardize and simplify it the Chinese government adopted in 1979 *pinyin* as the official romanization system. The *pinyin* system underpins the drive to establish a nationally-recognized spoken language. This system has been followed as far as possible in what follows. Certain spellings such as Beijing for Peking are now becoming familiar but Guangzhou for Canton perhaps less so. Shanghai remains the same, but the Yangtse river which enters the sea at Shanghai is now written Changjiang. The structure of Chinese personal names presents additional problems but two-syllable (two-character) components are often now written as a single word. Two of the most famous names, Mao Tse-tung and Chou En-lai, are now rendered Mao Zedong and Zhou Enlai. Hong Kong, which has some claims to retain this spelling at least for the present, has not been referred to as Xianggang, the *pinyin* romanization. □

other cryogenic gases on the industrial market, the suspicion must remain that the Chinese Academy of Sciences would have preferred to make its own, for fear otherwise of having to depend on other agencies, with different goals, for strategically important materials. These traditions of self-sufficiency have some advantages, not least in providing working scientists with a sense of the economic cost of laboratory supplies that might otherwise be taken for granted. But the diversion of creative people's time and energy to the simplest of laboratory tasks is a serious waste. Chinese who have spent time overseas are constantly remarking that their counterparts elsewhere can order supplies by telephone, and often expect them to be delivered the following day. Often, it seems easier for a Chinese laboratory to order an expensive piece of equipment from a foreign company (because the provision of funds for advanced equipment has been generous almost to a fault) than to spend a fraction of the cost buying goods and services within China.

The ever-present influence of the Communist Party is, on balance, another brake on effectiveness. But the issue is neither black and white nor immediate, as it may seem, from the West, it is bound to be. China's traditional respect for scholarship has ensured that the heads of institutions are invariably able people. The notion that for every such person there should be a party secretary, usually full-time, whose constituency consists in principle of the whole institution, but whose criteria for deciding what should be done may be qualitatively different, would seem a recipe for continual disaster. The Cultural Revolution shows that disasters on a national scale can happen. But for the present, the chief influence of the institutional party seems generally supportive of what the managers seek to do. So long as the cry is that science must do more to make China's industry prosperous, and while research directors share that aim, there is unlikely to be trouble. It is naturally impossible to tell how often original lines of enquiry are stifled by this unfamiliar consensus.

Survival

Against this background, perhaps the most remarkable feature of the research enterprise in China is that it has somehow been able to survive. Where else could half of a country's research workers have been compelled to work as manual labourers (or worse, imprisoned or simply killed) for between four and six years and then be able to return to pick up the threads of their old projects? It may be true that much of the research at China's research institutes and university departments consists of an attempt to replicate what has already been done elsewhere, but it also often has a distinctively Chinese flavour. People are anxious to learn other people's techniques, but the problems they intend to solve are their own. The success, at a host of China's laboratories, in identifying the active principles of traditional Chinese medicines, and the way in which the Institute of Genetics at Beijing has used the techniques of meristem culture to improve and propagate crop species not tackled elsewhere, should be a proof of that.

In any case, if shortcomings persist, they are now to be removed by means of the reforms decreed in March (see p. 222). That, at least, is what the officials in Beijing would like to think. In future, researchers will be able to succeed either by winning grants for the support of their research from a National Science Foundation still to be set up or by engaging in some kind of industrial activity. (Contract work for industrial enterprises will be commonplace; the search for innovations with industrial potential will be the more daring course, but sometimes people will turn their laboratories into factories.) Those who do not succeed in either course will keep their posts, their salaries and their apartments, but will be subjected to social pressure to do something, which is one of the ways in which the Party may help. So will this not powerfully contribute to China's goals?

Yes, but also perhaps no. That the new arrangements will increase the interest of researchers in China's industry is certain, but there is at least some cause to fear that China's industry may be so short of technicians and technically trained managers that the intervention of more researchers from academic institutions

may be an embarrassment. As things are, the proportion of young people entering higher education is about three per cent. Nobody will be surprised that there are too few engineers, and it is wise that the next five-year plan (to begin in January) should allow for more expansion of technical than of university education. But it will also be a surprise if, ten years or so from now, the then Chinese government is not bewailing the folly of its predecessors in neglecting the rapid expansion of higher education. China has done wonders in the past three decades, by making more than 90 per cent of its population literate: should it now adopt the goal of bringing out the full potential of that literate population? The government may have underestimated the scale on which higher education needs to grow.

Research

It may also have underestimated the importance of basic research to developing China. To be sure, the government is busy with high-energy physics and with space research. But much of China's interest to the outside world, and capacity to win its collaboration, rests on the high quality and the distinctive flavour of what is best in China's research laboratories. Natural cleverness helps. The tradition which China's science has recently cultivated of setting itself impossible goals and then attaining them (the synthesis of insulin is the best-known example) is potentially stimulating for the rest of us.

There are several jokers in this pack of cards. The contrast between mainland China and Hong Kong is not the consequence of an historical accident, but is a sign of what difficulties the slowly waking mediaeval state will have to face in a very few years. The mainland Chinese who now comply with regulations about where they may choose to live, or how many children they may have, are the same Chinese who have made a scattering of improbable islands into one of the richest manufactories in the world. When the mainland Marxists shake their heads over the objective realities of the Marxist dogma, they are talking theoretically; what will they do faced with the contrast between Chinese peoples' inclinations and their system?

Overseas relations are also important. China for the past decade has revered the United States and, by extension, the rest of the West. Now there is a grudging recognition that Japan is passably a success. In the end, it seems more probable that China will turn eastwards, not just to Japan but to the Pacific as a whole. But what if it made common cause with India, another continent from which it is divided by its common character — a huge and diverse population in the fertile sub-tropics, a gulf between the peasants and the rest, a sense of antiquity?

So how should the outside world respond to what is happening in China? The urgent need for training for young scientists has been acknowledged and partly met, but more generosity would be seemly, even prudent. A greater interest in what is happening in Chinese science would be worthwhile in itself, but would also help to strengthen China's self-respect. Too many of China's journals go unread. More visitors not so afflicted by culture-shock that they can say what is wrong in what they see would be welcome, if only as a mark of the adulthood of China's science.

For China, the immediate problems are economic and political. The need not to grow more than twice as fast as the rest of the world seems to have been appreciated. Whether China will be able to achieve the goal of being as prosperous as the West while retaining its communist ideals is both an economic question (what kinds of incentives will be how generally spread?) and, obviously, a political question. Technically, and by its talent, China's research community could easily provide what the government expects of it. Not only China's technical community, but the rest of us, have a vital interest in knowing how the economic and political questions are resolved.

This survey has been written by Alun Anderson and John Maddox, whose visits to China in July and September respectively were arranged by the Chinese Academy of Sciences (Academia Sinica). *Nature* is grateful to the academy, and to the directors and staffs of many of its institutes for their help. [

How China runs its science

A long chain of command

At the very top of the pyramid of organizations involved in science and technology comes the **Science and Technology Leading Group of the State Council**; essentially a group of members of the State Council with a special interest in science and the power to push important measures through. The State Council is the highest body concerned with the day-to-day running of the nation — it is the equivalent of the Cabinet in a Western country and is supervised by the Standing Committee of the National People's Congress. Members of the National People's Congress — the highest representative body — are elected every five years and in turn elect the Standing Committee and the Head of State, currently Li Xiannian.

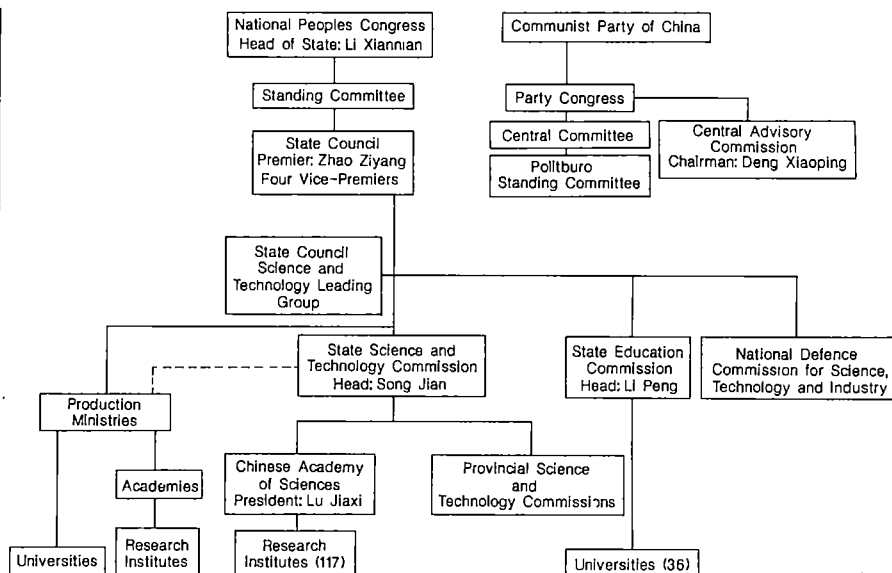
Although the State Council supervises all the ministries and agencies, as well as the People's Liberation Army through the Ministry of National Defence, it does not lay down fundamental policy. That job lies with the largest political party, the Communist Party of China (CPC), and in particular with the Standing Committee of the Politburo of the CPC. Almost 40 million people are members of the party, membership requiring, among other things, a willingness to dedicate a great deal of one's spare time to Party work. But the CPC is not China's only party. Eight others exist although they lack any real power.

Members of the State Council are frequently also members of the Standing Committee of the Politburo and, of course, most of those elected to the National People's Congress are members of the CPC. Not all Politburo members hold state office; Deng Xiaoping, the most powerful man in China, does not.

Immediately under the State Council comes the **State Science and Technology Commission (SSTC)** with responsibility for formulating and implementing science and technology policy within the guidelines given by the State Council. Overall planning and financial support are worked out in cooperation with the State Planning Commission and the State Economic Commission.

Under SSTC supervision but with a great measure of independence comes the **Chinese Academy of Sciences**, whose 117 research institutes carry out the bulk of China's basic research and are well known to academic visitors from the West.

Also under direct control of the State Council are the various ministries. One, the Ministry of Education, became the **State Education Commission (SEC)** in June of this year, a jump in status which reflects important policy decisions on education. Attached to the SEC are 36 universities including many of the most



prestigious "key" universities. But there are many universities which do not belong to the SEC but are attached to individual **production ministries**, for example, the Ministry of Petroleum has five universities.

In total, the number of ministries is very large by Western standards. Even though reforms in 1983 halved the number of high-level government bodies, there are still 33 ministries and 6 commissions (as against just 12 ministries in Japan). Just why there are so many is easy to understand when it is remembered that all the means of production are owned by the state. To administer and plan their activities a whole set of industrial ministries is required: aviation, chemicals, coal, machine building, metallurgical industry, nuclear industry, space, textiles and so on, just as in the Soviet Union. Many of these ministries have their own schools as well as universities; they may have their own farms and own factories supplying consumer goods for their workers. Each ministry is thus semi-autonomous.

Some ministries also have their own research **academies**; best known are the **Chinese Academy of Medicine** under the Ministry of Public Health and the **Chinese**

Academy of Agricultural Sciences under the Ministry of Agriculture, Animal Husbandry and Fishery. Each academy in turn runs several research institutes. Individual factories and state enterprises supervised by the various ministries also maintain research laboratories, some of them large.

At the provincial and municipal level of government, much of the above pattern is repeated on a smaller scale. A **Regional State Science and Technology Commission** coordinates local research efforts and bureaus of the local government which match the central ministries look after local industry and solve their technical problems through small research institutes.

Almost all basic research is carried out by the big national institutes: the Chinese Academy of Sciences, the "key" universities, and the academies belonging to ministries.

The large number of scientific societies in China is coordinated by the **China Association of Science and Technology (CAST)**, an organization of growing importance not only in arranging academic meetings and international exchange but in its educational activities and in the expert advice it gives to state bodies. □

The new reforms

Pragmatism now the watchword

DRAMATIC changes are taking place in the organization of science and technology in China. The changes will affect all scientists, in the universities, research institutes and in industry, and will reward those institutes and individuals that contribute to China's scientific and technological development.

This development is no surprise. The reforms are themselves a reflection of the huge changes in social and economic life that have taken place under the pragmatic leadership of Deng Xiaoping. The reform began in China with the smashing of the Gang of Four and Deng's return to power in August 1977. By 1982 and the 12th Par-

ty Congress, he and his supporters were in complete control and the rhetoric and personality cult of Chairman Mao and his successor, "Wise Leader Hua", had been cast aside.

National goals now seem to have less to do with ideology than with making rapid material progress. The primary aim, set by the 12th National Congress, is to "quadruple the gross annual value of China's industrial and agricultural output by the end of this century and then proceed to raise, within the first 30–50 years of the next century, China's economy close to the level of the developed countries".

To achieve these goals, Deng has trans-

Individual responsibility taking over from egalitarianism?

THE "responsibility system", which has lately given Chinese peasants the right to sell food crops for what they can make in the free markets, also applies to the research enterprise, but in ways that seem to vary in a bewildering fashion from one institution to another. This is why the State Council's Leading Group on Science and Technology is even now drafting regulations to determine what should happen to the financial rewards of greater collaboration with industry. Wu Mingyu, vice-chairman of the State Science and Technology Commission, hopes that the rules will be ready by 1986.

At present, the practice is that a sizeable proportion (usually a half) of the money received under industrial contracts is passed on to the parent organization, the Chinese Academy of Sciences or the Commission on Education. It also seems that individuals can receive between 5 and 8 per cent of the income earned from industry by way of higher salaries, although these awards will certainly be shared by the whole of the research group concerned, perhaps the whole of a department and sometimes even by the whole staff of an institution.

Thus at the chemistry department of the University of Beijing, 30 per cent of outside income is handed over to the university and

rather more than 40 per cent goes on further development (which means research equipment and consumables). Personal awards amount to less than 10 per cent of the total, and are shared out among the whole of the department's staff *pro rata* with salary.

There is some chagrin at the high rate of tax on such payments. Annual supplements of less than three months' salary are paid gross, but if the amount of a person's extra payment should be 6 months' salary, the department is required to pay over three times as much to the state, thus sharply reducing the total available for awards.

At the Academy's Institute of Microbiology, the practice is different. Outside income is shared equally with the Academy, while 30 per cent of the receipts is used to cover research costs (so reducing the recurrent cost to the Academy). Between 5 and 8 per cent of the institute's share (roughly 500,000 yuan last year) can be used for making awards to individuals responsible for the research concerned, while the remainder is spent on project-related research.

Elsewhere, the novelty of the notion that research can spell money means that for the time being much more informal arrangements obtain. At the Institute of

Electronics at Guangzhou, which has just sold the software for a word processor program for manipulating Chinese characters to a Hong Kong company, for example, the local branch of the Academy seems not yet to have learned about the contract, and thus has not had a chance to say what should be done with the HK\$ 100,000 the institute now has sitting in the bank.

The rules now being drafted are likely to endow these arrangements, themselves quite novel, with a degree of formality. Wu Mingyu says there is a case for treating institutions differently according to the balance of their research, basic or applied. The obvious difficulty is that under the system to be introduced in 1986, when basic researchers will be expected to compete for support by means of formal applications to the National Science Foundation (see p. 210), the pattern of financial pressure will still leave even the best basic researchers with the knowledge that they would be financially better off working on a contract for an enterprise of some kind.

The obvious resolution of the difficulty would be to include salary supplements in grant awards. It will be interesting to see whether the leading group decides that some such solution, however inequalitarian, would be in China's best interests. As things are, it seems, anything is possible. □

formed Chinese life: the door has been opened to the West to encourage foreign investment; market forces, not central planners, are to determine the pattern of industrial production; peasants are allowed to farm their own land and to sell their surplus produce at free markets; and intellectuals and scientists have been given back their high status but not yet salaries to match. At the same time, many leftists who were promoted during the Cultural Revolution are being removed from office; the Peoples' Liberation Army is being severely reduced in size.

But the path to reform has not been uniformly smooth. In September, at a national conference of the Chinese Communist Party convened exceptionally between party congresses, Deng had to pay a high political price for his latest triumph, the replacement of more than sixty ageing members of the Central Committee by younger people, some of them conspicuously Deng protégés. For one thing, he found it necessary to emphasize in his own speech to the conference that reform should not be allowed to jeopardize Marxist principles, while the delegates also heard a sombre warning from another member of the Politbureau, the veteran Chen Yun, that the opening of China to the outside world, necessary though it may be on economic grounds, entails the risk of "corrosion by decadent capitalist ideology and conduct". Chen's speech was widely regarded in September as a demand that the reformers should be

more alert to the dangers of the course on which they had embarked.

In his capacity as first secretary of the Central Commission for Discipline Inspection (an organ mirrored at all levels of Chinese party society), Chen went on to demand that the Party should be "vigilant" in the fight against lawlessness, corruption and pornography. The "bourgeois decadent ideology of putting money above all is corrupting party conduct and social conduct in general", Chen said.

This is one sign that opposition to the process of reform, although now largely silenced, has not entirely vanished. Now, it is as if the process of reform itself is on trial. Its successes have been spectacular, especially in the countryside, but there have also been problems galore. The official statistics show that China is now entering its fifth consecutive year of growth at ten per cent a year or more, which is amply reflected both in the construction sites being worked in all the cities and by ordinary people's interest in consumer goods (refrigerators for the time being the most sought after). The other side of this coin is that imports are growing faster than exports, while many of the new projects on which the reformers have embarked have come to grief. These are now some of the reasons why the outline of the next five-year plan (1986-90), also agreed at September's national party conference, offers the more modest target of 7-8 per cent a year — twice the present rate of growth in Western Europe and still

enough to reach the target of quadrupled production by the end of the century.

On a more human scale, new opportunities for people to make money have led to some spectacular cases of corruption, even involving Party members. Some changes have had to be undone abruptly. Thus, in 1984, fourteen coastal cities were declared special economic zones, free ports of a kind in which foreign investment would be encouraged and in which, it was intended, joint enterprises between Chinese and overseas companies would flourish. By July this year, however, all but four of the economic zones had been closed, partly because they had not attracted suitable investment, partly because of the fear that corruption would get out of hand.

Inevitably, the rapid growth of the past five years has brought great inequalities. Perhaps for the first time in Chinese history, there are people in the cities who envy the peasants. The official response that "some must get rich first" does little to ease the tensions that have built up.

When the new pragmatic policies first surfaced in 1977, science and technology were seen as a force that could transform China. Soon science was to obtain a place of honour among the "Four Modernizations" (science, agriculture, industry and defence). And after years of being reviled among Mao's "stinking elements" (including landlords, capitalists and counter-revolutionaries), scientists and intellectuals suddenly became respectable again.

In 1978, Deng announced that "intellectuals are part of the working class", professional titles were restored and academic societies allowed to function again. In the euphoria of this "Springtime for Science" it was not surprising that over-optimistic plans were laid.

At the 1978 National Science Congress which confirmed the restoration of science, huge programmes were outlined in eight areas: agriculture, energy, materials science, computer science, lasers, space, high-energy physics and genetic engineering; and plans were included for a 50-GeV proton synchrotron and a fusion machine on the same scale as the Joint European Torus (JET). But within a few years, the limits of what could be achieved were realized, and attempts to aim straight for high technology were modified. On 13 March 1985, a new and thoroughly pragmatic series of reforms were announced by the "Decision of the Central Committee of the Communist Party of China" after some years of discussion with scientists.

The essence of the reform, entirely in line with general policy, is that "science and technology must be oriented to economic reconstruction". To that end, "the defect of relying on purely administrative means in science and technology management, with the state undertaking too much and exercising too rigid a control" must be remedied. The plan says that while research projects of national priority should remain under the control of the state, other activities conducted by the scientific and technological institutes are to be managed by means of "economic levers and market regulation". In other words, many scientific institutions and scientists are going to have to find customers to pay for their work. Many defects of the present system are frankly described in the decision document, and the ways they are to be remedied are made clear.

First, the system of the "great iron pot" is to be eliminated. Hitherto, the huge numbers of research institutes attached in one way or another to the government have yearly been doled out fixed sums of money for their services, largely calculated according to staff numbers. The key projects on which they are to work have also been determined by the government and allocated to institutes deemed suitable to carry them out. The result has been research institutes that are isolated not only from industry but also from one another, under little pressure to deliver results, and seriously overmanned.

Under the new regime, state handouts for research are gradually to be withdrawn. Instead, institutes are to "earn income and accumulate funds" by contracts, by transferring research achievements (to industry), operating joint venture and export (companies), and consultancy. The experiment has already begun. Some research institutes have seen their state income cut; soon the system will spread nationwide. The Party expects that some

institutes will not survive in the new competitive atmosphere and will have to close. Others may find it expedient to merge with related institutes or even with industrial organizations. All institutional innovations, it seems, will be encouraged.

Institutes carrying out basic research will come under a different kind of pressure. It is accepted by the Party that basic research must continue and that financial support cannot come from industry. But the costs of basic research (over and above the salary costs of those responsible) will no longer be provided automatically with the public subvention for an institute. Instead, there is to be a system of competitive grants open to all scientists in China, not only those who work in formal research institutes.

A start has been made on the development of such a system within the Chinese Academy of Sciences, which has set up a small science foundation to receive grant applications for researchers at its own institutes. These are subject to peer review, as elsewhere. Next year, according to the plans now laid, there will be a National Science Foundation for whose funds scientists from universities will be able to compete on an equal footing with those at research institutes operated by the production ministries and provincial governments as well as by the Academy.

As recently as early October this year, the constitution of the new foundation had not been finally settled, although there is every likelihood that the State Science and Technology Commission will have formal charge of it. But officials say that the chairman of the foundation will have to be an independent person. The scale on which the foundation will operate is similarly undecided, although it seems clear that an annual budget of less than several hundreds of millions of yuan a year will mean a cut in basic research support.

The new system will have radical consequences for the ways in which individual laboratories function. In many of them, people whose work can attract support from industry will be working alongside those who must look to the National Science Foundation for help with research costs. Even when grant applications succeed, basic researchers will not be entitled to the salary supplements that those working on applied research projects may in future enjoy when industrial innovations are taken up and exploited by industry.

Some fields will continue to be supported differently. Research institutes involved in medicine, public health, family planning, disaster prevention, meteorology and so on will continue to be supported by annual grants, but the plan is that the institutes will in future make proposals indicating which projects they wish to carry out. This is a departure from the present system, under which projects have been allocated from on high.

To get the institutes in shape for the new competitive world, they will be given con-

siderable decision-making powers of their own. During the Cultural Revolution, research institutes were run by "revolutionary committees" dominated by Party officials; ideology took precedence over scientific goals. Now, the power of scientific directors is being restored. Party officials are "to help with the effective application of the system under which directors assume overall responsibility and promote scientific and technical development". Indeed, a prerequisite for a Party committee secretary is now that he or she should "possess a certain level of scientific knowledge and be keen on scientific and technical undertakings and conscientious in carrying out the Party's policy towards intellectuals", a far cry from the days of the Cultural Revolution. Directors will thus now be firmly responsible for the scientific direction of their institutes and can, in principle, even sack inefficient workers.

There is also to be more freedom and greater rewards for the individual scientists. They will be allowed to do part-time consulting work and to keep the money they receive from it (minus any sums due for the use of state facilities). Large rewards are to be made to the individual scientist (rather than the institute) for major achievements (although there may well be pressure to share any such rewards out). And hitting at one of the most intractable problems of the present system, "rational job mobility" is to be introduced for "competent people must no longer be made to sit idle and waste their talent".

How far this reform will go remains to be seen; there are already semi-official newspapers in circulation offering job appointments; some show a huge range of perks for those willing to take their skills to organizations outside the big cities. But the present system of allocation to one's place of work upon graduation is still far from anything like an open job market.

What are the chances that the reforms can be put into practice? Accounts of how individual research institutes and universities are coping will be found in the pages that follow: the pace is faster in some than others, and some new kinds of profit-seeking technology corporations, unthinkable a few years ago, have sprung up. But China is a vast and bureaucratic country and changes at the lower levels of the hierarchy do not automatically follow central directives at any speed.

A story is already in circulation of a scientific director of a small and distant research institute who found, despite Party directives, that he could not wrench control over research from the local Party man. In the end he travelled to Beijing, appealed to Party authorities and returned with a high official who made it clear that the Party line was to be followed. No doubt change will be resisted at many levels by many different forces: at this early stage it is hard to guess what will emerge in the next year or two as the reforms take effect. □

Science policy

Low versus high technology

OVERALL responsibility for China's science and technology policy rests with the State Science and Technology Commission (SSTC). Like every organization associated with science, its fortunes have changed with the times: it was set up in 1958 during the Great Leap Forward, abolished at the start of the Cultural Revolution, and re-established in 1977 after the fall of the Gang of Four.

Since then the influence of the SSTC has gradually increased, at first under the leadership of Fang Yi, a very experienced politician and member of the Politburo. He did much to establish the SSTC's political respectability, but although he was college educated (unusual in the upper ranks of the Party), he had never been a research scientist. Now, the job of director general has gone to someone with a weighty scientific reputation: Song Jian, a 53-year-old cyberneticist, who trained in the Soviet Union, studied in the United States and took a leading role in the successful development of the space industry.

Most of the SSTC's departments are concerned with national policy, planning and legislation in science and technology. One of them, that for science and technology policy, effectively acts as a think tank for the Science and Technology Leading Group, the group of senior politicians in the State Council with special responsibility for making sure science policy matters receive top priority. And the SSTC also exercises ultimate control over the Chinese Academy of Sciences and ensures that government policy is carried out in a coordinated manner at other research facilities.

The SSTC does not itself maintain any experimental research facilities. Nor does it control directly the major part of the science budget. But it can combine the use of its own funds with the influence it has over allocations arrived at by the State Planning Commission (and given out by the Ministry of Finance) to provide effective control.

What this process has meant until now is the SSTC sitting down with the various science-related ministries and deciding on key projects of national importance that need special promotion — like those for

the development of the Yellow Loess Plateau, and fish aquaculture, for example — and then giving guidelines for how the funds should be allocated by the Ministry of Finance (on top of any routine allocations) as well as giving out the part of the budget it controls directly.

With the new reforms, much of this will change. The commission will still come up with key projects, but, as Lin Zixin, secretary-general of the SSTC put it, "instead of us selecting, we would like to let bids come from research organizations. Research, even on key projects would then be carried out under contract". Experiments are just beginning to be made with this new system, and if it turns out to be successful, then inefficient research organizations, unable to gain funds, will wither away. To sum up, "we used to choose from the top, the new plan is to be the reverse"; and at the same time, the new National Science Foundation will "cover a portion of basic research and applied research that can't produce practical economic results quickly".

This pragmatic approach comes along with a re-evaluation of what China can really achieve. Back in the heady days of 1978 many people were ready to believe that China could leap straight into an era of high-technology and catch up with the West.

Indeed, there are plenty of areas where China has done extraordinarily well: the manufacture of satellites, H-bombs and high-power electric generators are no mean technological achievements. The problem is that basic items such as bicycles, sewing machines and radio sets are of low-quality. Now there is an awareness that success in a few areas of high technology does not automatically feed into everyday products. Thus the SSTC has laid down five clear principles:

- to develop technology coordinately with society so that society can absorb its fruits and provide for its support;
- emphasize production technology;
- develop new technology in the industrial enterprises;
- in basic research stress China's unique problems;
- increase international cooperation.

These principles may sound unsurprising but they represent radical breaks with the past. Emphasis on research inside enterprises is a clear rejection of the Soviet model — independent state-controlled research institutes — which has been followed since 1949; instead it is now Japan, whose companies perform their own research, that provides inspiration. And emphasis on international cooperation is a complete break with the view held during the Cultural Revolution that "learning from abroad is a betrayal".

One problem still to be tackled is how to

get sufficient talent into industry — it will mean countering the prestige of the Chinese Academy of Sciences, for one thing. As Lin put it, "we have never paid enough attention to the needs of the enterprises, the best graduates were allocated to the institutes and government agencies. Although there is no difference in pay between factories and academia, people feel they are at a lower level if allocated to a small factory. This is a complex social problem which is very difficult to change". But the carrot of financial incentives for those who work in industry is not yet under consideration.

It is clear that there is still debate over what technological level China should be trying for with some still believing that the "highest" technology is the best technology. Lin holds the view that it is selecting appropriate technology that is important. For example, in 1978 Chairman Hua



The State Science and Technology Commission's headquarters.

announced that 85 per cent of agriculture would be mechanized by 1988 — not a prediction that will be fulfilled. But Lin now argues that the use of draught animals and manual labour is actually much more rational; it should not be thought of as a sign of backwardness.

The planning of basic research is largely to be left to the Academy. But there will be some emphasis on solving problems specific to China. One example is research on rare earth elements in which China is particularly rich, and on which much basic research in refining remains to be done.

But whatever policy decisions are made there remains the awareness that shortage of technical skills, more than anything else, will determine the pace at which technological progress can take place. There are, according to the SSTC, 1.6 million enterprises in China but only 1.8 million "technical" staff who have received post middle-school training. In the electronics industry only 20 per cent of staff is trained, in the iron and steel industry 10 per cent, and in textiles and light industry 1 per cent. It is boosting these figures that will have to receive the highest priority of all. □



Lin Zixin, secretary general of the State Science and Technology Commission.

Academy of Sciences

A pawn in revolutionary history

THE history of the Chinese Academy of Sciences is a mirror to the history of New China; for better or for worse, each change in policy has affected the Academy's fortunes. The organization was founded in November 1949, just one month after Mao Zedong proclaimed the founding of the People's Republic in Tiananmen Square. First priorities were to build an effective scientific research community; the Academy began with just twenty institutes and 300 research staff inherited from pre-revolutionary days. A major source of help was scientists who returned from overseas inspired by the desire to help China's development. A new science press was soon established and a major library; branches of the Academy were built up throughout China and academic relations with foreign countries — mainly in the Eastern bloc — were established.

In this early period China astonished the world by the moderation of its policies. Land was redistributed to the peasants and factories gradually nationalized, with compensation paid to the owners, giving China the highest growth rate in Asia.

In 1956 enthusiasm for science intensified when Mao Zedong called for science to lead the country forward and gave freedom to intellectual enquiry with the famous slogan "Let a hundred flowers bloom, let a hundred schools of thought contend". A 12-year science plan was also made with emphasis on natural resource exploration and on catching up with the West in key technologies — atomic energy, semiconductors and jet aircraft. All this helped to generate enthusiasm for science among the people.

But that enthusiasm was soon to get out of hand. In 1958 the Great Leap Forward began. Mass enthusiasm for science led to wild optimism about what it and China could achieve. The slogan was "Catch up with the USA, overtake Britain". As revolutionary fervour was intensified by mass campaigns, millions of peasants were reorganized into huge communes, massive irrigation projects begun, and dams built. The effects were felt in the laboratories; normal work ended, wild claims were made for all sorts of fantastic inventions and many people spent their time trying to put into effect the revolutionary sayings of the Chairman rather than doing research. In some laboratories the "backyard blastfurnaces" that Mao had claimed would allow rapid modernization were set up. And those who had spoken out during the "hundred flowers" period now found themselves under attack as rightists. Overall there were some gains for science in the enthusiasm for it which spread among the people, but the disruption prevented any real progress being made.

The dominance of the radicals ended as the Great Leap Forward ground to a halt in a morass of inefficiency compounded by some of the worst natural disasters China had experienced for a century.

In 1961 many tens of thousands of peasants starved to death in the countryside. And the year before, China had finally broken with the Soviet Union over the latter's "revisionist" policies and "friendship" with the United States, resulting in withdrawal of all technical advisors.

These disasters were quickly followed by a swing from the radical to more pragmatic policies. Mao Zedong lost his post as Head of State to Liu Shaoqi and Deng Xiaoping — now the most powerful man in China — became Secretary-General of the Party. These two, along with Premier Zhou Enlai did much to undo the worst aspects of the Great Leap Forward; some private plots of land and financial incentives were introduced in order to improve efficiency.

For the Academy it was a period when the role of scientists was made clearer. Guarantees were given that scientists could spend five out of six days in their laboratories (instead of at political classes); gone was the time spent on writing poems to praise Mao, practising in the militia, performing manual labour along with the peasants and all the other things that participation in mass movements had required. Also it had become clear after almost ten years of turmoil that there simply was not enough food for the Chinese people. Agricultural research now proceeded at top speed and real gains in food output were made. By 1964, science was flourishing as never before. In 1965 the Academy succeeded in launching a satellite, the world's first chemical synthesis of insulin was completed at the Shanghai Institute of Biochemistry and major advances were made in atomic energy research. But then the Great Proletarian Cultural Revolution began.

For some years leftists associated with Mao Zedong had been attempting to regain power through a Socialist Education Movement intended to mobilize the peasants to greater revolutionary ardour. Then in July 1966, after more than a year in which he had made no public appearances, Chairman Mao, then 73 years old, was seen in television pictures broadcast worldwide taking a "ten-mile" swim in the Yangtse river. The next month the Red Guards appeared in strength. Deng Xiaoping and Liu Shaoqi fell from power, the latter to be branded a "renegade, traitor and scab" and to die in disgrace. Deng spent two years in solitary confinement before being exiled to Jiangxi Province.

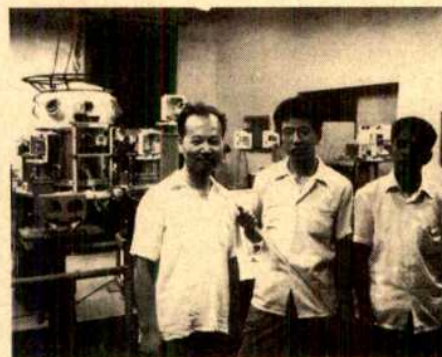
The ten years that were to follow were

Leading light in lasers

ONE of China's laser researchers remarked that there are more people studying lasers in China than in the rest of the world put together. Although said with a laugh it might even be true; one problem in China is that too many scientists cluster around a fashionable topic (a phenomenon dubbed the "swarming of bees") and then, because institutes are isolated from one another, proceed to duplicate low-level research being done elsewhere.

The Academy's Shanghai Institute of Optics and Fine Mechanics is not among those doing low-level research; it is at the top of the laser tree. It is not actually in Shanghai but in Jiading, more than an hour's drive out in the country where it was built in 1964 as the first part of a science city. The Cultural Revolution then came along however and the city was never completed.

The institute now has 600 researchers, 500 technicians and administrators and some worthy achievements. Among them are the development of inertial confinement fusion systems, a three million joule per pulse high-energy laser, and excimer lasers. Basic research at levels no different from that in the West is going on in laser



Professors Fang Honglie, Bi Wuji and Wang Kewu (left to right) stand in front of the target chamber for their six-beam, 10-watt a pulse, inertial confinement fusion research machine.

spectroscopy and atomic hyperfine structure. But even so the institute is under strong pressure to earn its own keep.

This year its funds were cut by 23 per cent and further annual cuts of 20 per cent are expected. The institute has reacted in two ways. First, industrial contracts are being sought and manufacturing facilities established. Fortunately the institute grows very good Nd laser rod glass as well as Yag, which has already been exported to Australia. Other commercial possibilities lie in laser writing of integrated circuit masks and medicine. Second, the institute is seeking "open" status: extra funds will be received from central government to allow scientists from all over China to come and run experiments, in a scheme administered by a national committee. And of course, institute scientists will be applying for funds from the new Science Foundation. □

Academy's computer centre really means business

THE "Technology Service Corporation of the Computing Centre of the Chinese Academy of Sciences" could do with a new title, as its general manager Yi Hui-en readily agrees. For far from being some sub-section of a research institute, the corporation is really a "high-tech venture business" of a kind unimaginable in the China of a few years ago.

The corporation began life in the summer of 1981 after the policy shift to a "commodity-based" economy was announced. Its aim was to train technicians who would then liaise with scientists in the Academy's research institutes and then help turn results into marketable products. Initial capital — just 50,000 yuan — came from the Academy. Senior middle-school graduates were provided to staff it, for at that stage an allocation of university graduates could not be obtained.

Despite these rather unpromising beginnings, the corporation has since generated 5 million yuan in profits and long ago paid off its loan. Now, not a single yuan is received from the state; everything including operating expenses and salaries is paid out of profits. And the profits ratio would make many a Western capitalist green with envy — last year profits of some 1.6 million yuan were achieved on a turnover of just

4.6 million yuan.

The corporation was one of the first of its kind in the field but there are now more than 200 similar organizations nationwide. All have the dual aim of "turning research results into productive forces" and "maintaining technological expertise, particularly through the training of technicians".

This particular corporation has made its money by catching onto the computer boom; others operate in fields as diverse as cell culture and new materials. Its biggest financial business is selling hardware, mostly small computers, but many of its 170 staff are designing software packages. It has set up a joint software venture with a Japanese company, is operating five factories in the Special Economic Zone bordering Hong Kong where a micro-computer is assembled for a US company and is also manufacturing automation control devices (programmable counting devices and the like). Now it is diversifying with a factory to produce vitamin oil by a method developed in Academy institutes in Hefei.

The corporation's staff claim they have complete freedom to launch new projects and choose new areas to explore. Ideas come from its own "think tank" containing scientists and engineers from various insti-

tutes. It is not restricted to working only with the Academy and links are formed with any research institute that might be able to contribute a marketable product. Diverse links are thus possible and it is hoped that more joint ventures will be launched with foreign countries. Now the corporation is a success, university-trained staff are being allocated to it for further training, and it may also be given the right to confer higher degrees.

Right now the only obstacle to further growth is lack of capital. But these are perhaps the golden days. The market is still firmly in the sellers' hands — such is the shortage of expertise that the corporation never needs to go out of its way to advertise its wares. But the prospect of growing competition for customers with other corporations is on the horizon.

Where then will be the boundary between communism and capitalism? No specific answer seems to exist. Perhaps the best that can be done is to quote Manager Qiao, a fictional character whose adventures were serialized in the *Workers' Daily* a few years ago. "Of course", Manager Qiao says "competition between factories in China will be totally different from the cut-throat struggle in capitalist countries. . . we can't scrap socialist cooperation". □

the worst period for science and technology. At the outbreak of the Cultural Revolution the Academy had 106 institutes, by 1973 the number had fallen to 53. Some were simply closed altogether, others were taken over by regional authorities and others packed up and moved far into remote regions of China. Research staff had stood at 60,000 in 1965; by 1973 only one-third were left. Some 30,000 scientists were sent for re-education in "cardre schools" in the countryside. Those who were simply given physical labour in the country were lucky; in some institutes more than half the staff died as attacks on any kind of intellectuals intensified. In one institute a political appointee claimed that "knowledge of a foreign language" was sufficient evidence of being "a foreign spy".

But the terrible sufferings undergone by scientists and intellectuals during the Cultural Revolution are already well known. Mention of these days is now almost always greeted with laughter — not because there is anything funny to recall but because a laugh is a standard Chinese response to a memory that is too painful or too embarrassing to talk about.

The Cultural Revolution proper lasted only until 1968, for by then the activities of the Red Guard had grown so uncontrollable that industrial production was being disrupted. The People's Liberation Army, headed by Lin Biao, restored order and sent millions of young people to work in the countryside and to establish communes in remote border regions. But the

persecution of intellectuals and domination by the left was not to end so quickly. In 1971 Lin Biao died in an "aeroplane crash" after a failed attempt to assassinate Mao Zedong. Only gradually, under the influence of Prime Minister Zhou Enlai, were things restored to something like normal. In 1973 Deng Xiaoping was once again given power.

His return was to be for a few short years in which there was not time to undo the harm of the Cultural Revolution. At the same time as his reappointment the "Gang of Four", headed by Mao's wife Jiang Qing, had launched a leftist campaign. Then in January Zhou Enlai died, removing the last remaining moderating influence upon the radicals. A few months later Deng Xiaoping was again dismissed from power. It was then that fate intervened — in September 1976 Mao Zedong died. Within weeks the Gang of Four were under arrest and the pragmatists were back in power. By August 1977 Deng Xiaoping was in high office for the third time.

Although the worst excesses of the "leftists" were over by 1973, very little progress was made in rebuilding the scientific framework between 1973 and 1977 while the leftists and the Gang of Four had influence. But after the "smashing of the Gang of Four" (a standard phrase in China, and one always enunciated with particular relish) progress was rapid.

In 1977 the "Four Modernizations" were announced as the key objectives for the nation: modernization of agriculture,

industry, defence and science and technology. A national science congress was convened in 1978 and a highly ambitious eight-year plan approved which would once more make science and technology a major force in the nation. Some of the more ambitious projects have since been abandoned or scaled down, as described elsewhere.

By 1985 the Chinese Academy of Sciences was in better shape than ever before, with 117 institutes and 80,000 staff. Almost half are technicians and scientists and the remainder administrators, support staff and Party workers. A huge support staff is necessary partly because the Academy has to manufacture much of its own equipment at its eight factories — it even produces its own gases for use in research laboratories as there is no national supply facility.

Institutes dispersed during the Cultural Revolution have in the main returned to their old homes; an exception is the Academy's university, the University of Science and Technology of China, which was moved to the small city of Hefei in Anhui Province. A science city has grown up around the university, including a number of new research institutes, and there is now no longer any possibility of moving it back to Beijing.

Like other institutions, the Chinese Academy of Sciences is now busy implementing the new reforms. Several unique moves have been taken to overcome the difficulties spelt out in the reform document. To answer the criticism that

institutes are too isolated, some, or some subsections of them, are to become "national" laboratories open to other research workers throughout China. The idea is that a part of the staff will not be fixed but will be appointed from other institutes on one- or two-year contracts. A national committee with representatives from many institutes will appoint the director of the new "open" laboratories. Seventeen institutes have already been chosen for the scheme: they include the Theoretical Physics Institute and the Institute of Mathematics. Many other institutes are also hoping to gain "open" sta-

tus, in part because funds will be provided by the central government which could help offset income lost as the reforms begin to take effect.

Another move which the Academy has been taking over the past few years is to establish new high-technology corporations which can market ideas developed in the Academy's institutes; one, described in the pages that follow, is principally concerned with selling hardware and developing software; others have spun off from botany into cell-culture technology, and from chemistry into the manufacture of new materials. □

Education commission and universities

Catching up the priority

EVER since the founding of the People's Republic, it has been the Chinese Academy of Sciences, not the universities, that has dominated the nation's basic scientific research. In terms of facilities, time available for research and funds the universities have been poor cousins to the Academy. But the new reforms and the rebirth of the Ministry of Education as the more powerful State Education Commission (SEC), are likely to give the universities a more equal chance.

Commission status was gained in the middle of June this year and in July, when a visit to the commission was made, the paint on the new signboard outside the building was scarcely dry. New policy decisions had not yet been made in any detail. One thing that was clear, though, was that the new head of the commission, Li Peng, is a powerful figure.

Li is one of the nation's four vice-presidents and holds a number of other ministerial appointments, including responsibility for energy. In September he was one of the "new generation" to be appointed as a member of the 24-man Politburo. Many believe he is on the way to the very top and at 57 he is still young in Chinese terms. Li is likely to be able to get things done and to be able to overcome bureaucratic resistance to some much needed reforms.

Why university researchers feel reform is necessary can be seen by a glance at the

table shown below.

Although the Academy takes a share of the state budget for natural sciences research many times larger than that taken by the SEC universities or universities attached to other ministries, it does not win the majority of State Science and Invention Prizes. Nor does it have a majority of highly qualified staff. Of course, neither of these measures can be said to unequivocally measure scientific ability, but the disparity in resources allocated to the universities and the Academy has been a source of friction for more than 30 years.

From next year, university scientists should get more of an equal chance to prove their worth. A National Science Foundation will be established in 1986 (although who will run it has yet to be settled by the State Council) and university researchers will then compete for grants with researchers from the Academy and elsewhere. They will also be able to bid for contracts for large research projects that would formally have been allocated by the government. The new foundation will probably have around 100 million yuan at its disposal for the first year, three times the funds of the present Academy foundation.

Bringing about changes of this kind will be just one task for the SCE, and a fairly minor one at that. More important is that the whole of higher education be managed

rationally. At present the SCE has 36 universities under its direct control, many of them "key" universities which attract the most funds and the best students. Other universities belong to other ministries; some of the best of these (for instance the University of Iron and Steel Technology of the Ministry of Metallurgical Industries) are managed jointly by the SCE and the relevant ministry, but most of them are exclusively managed by their own ministry. Graduates from these latter universities — or around 80 per cent of them — are sent to the parent ministry's offices, colleges and factories to work. Only the remainder may go elsewhere.

No-one feels that this divisive system is really for the best; but change is sought that goes beyond just making all universities answerable to the SCE. Instead, the Party wants to see a loosening of links between all universities and their ministries, including even the SCE; just as there should also be a loosening of links between factories and ministries. Indeed, going higher up the hierarchy, the Party would like to see many ministries done away with and turned into state corporations. A few years ago, that was what happened to the Ministry of Shipbuilding; it became the Shipbuilding Corporation to "overcome bureaucracy and to practise free competition".

How far reform can go in the case of the universities is hard to predict. Many academics are sceptical, one commenting that "it cannot be changed", but then adding "but maybe Li has the authority to do it".

Li will also have his hands full in simply trying to get the whole educational system close to world standards. In 1981, there were around 1.279 million students at China's universities, 1.061 million of them at four-year universities and the rest pursuing two-year courses. Now the total is close to two million. In the Philippines, however, with a population of just 48 million, there are 1.8 million university students. Things are certainly getting better and this year, half a million university places will be available for three million applicants, but there is still an enormous distance to go.

Further down the system problems are grave. Before university comes three years senior middle school, three years of junior middle school and six years of primary school. The goal being fought for at the moment is universal primary school education. Only wealthy Jiangsu Province has so far been successful. Nationwide, 94 per cent of children enter primary school, but only two-thirds complete the first six years of schooling, with drop-out rates very high in rural areas. The hope is that primary school attendance will be made compulsory by 1990. One problem is that education, even at the primary level, is not free and many peasants want their children at home working on the land. □

The natural sciences in China 1984

	SEC universities	Chinese Academy of Sciences	Other universities
Full and associate professors	10,765	2,956	26,299
Doctoral students	316	140	233
MSc. students	13,207	2,885	16,815
Science and Invention Prizes	163	152	112
Central government budget (million yuan)	20 (160)*	460	30

*Including funds from industry for research in natural sciences. The above figures do not include salaries or major capital expenditures.

Life as a student

THE exact conditions that students face at university vary from place to place: some have introduced Western features in their curriculum and assessment methods, others adhere to a Soviet model and a minority have not yet recovered from the effects of the Cultural Revolution. But political classes, which once dominated university life, are down to 72 hours a year in the key universities.

One constant factor seems to be that all students live on campus, generally six to eight to a dormitory with bunk beds and one long table and bench being the norm. At graduate level, more space is provided and students live two to three to a room.

At Qinghua University a US-style grade system has been adopted. Each term the available courses, teachers and hours of lectures are announced along with the number of credits that can be gained towards the total needed for a degree. The course is five years, rather than the usual four, and a final-year project is seen as of key importance. That project might be something like taking an old theatre and making a plan for its restoration. Once all that is completed, the student faces an oral exam: 20 minutes to describe the project and then 40 minutes to defend it under questioning from the staff.

Entry into graduate school is not, however, gained by obtaining a good degree. A quite separate nationwide examination is taken in the winter of the final year, with the results announced in April. The successful student does not have to stay on at the same university for graduate work but 70 per cent do so.

Those who do not go on to further studies will be assigned jobs on graduation. In the old days (just a few years ago), students had no choice over where they would be sent to work. But nowadays lists of jobs are usually circulated, with the best students given first choice. The lists are prepared by the State Planning Commission after meetings at provincial level to collect requests for the highly trained products of the key universities. Once a student has been appointed to a work place, he or she may have little chance of changing it. The system seems widely accepted by students (as is the extreme difficulty of obtaining permission to live in Beijing or any of the big cities) as being necessary for the "good of China"; but that does not stop people using every trick in the book to make sure it is someone else who is sent to a small factory, far from the capital.

Yet another examination awaits those who wish to go on from graduate school to a doctoral course. But those who do go through and complete the doctoral course are virtually assured of a lectureship: as doctoral courses began again only a few years ago, there are still only a few hundred with such qualifications in the whole country. □

University entrance

Cramming to join the elite

THIS year around half a million university places will be available; more than ever before. But with three million students likely to be taking the entrance examination, that still means tough competition, especially for entrance into the elite key universities.

For universities at the very top like Qinghua, Fudan and Beijing, successful applicants will need to achieve a score of somewhere around 455 out of 600 (76 per cent) in the six-paper, 100 marks a paper entrance examination. Papers can be taken in physics, chemistry, biology, history, geography, Chinese, English, mathematics (with separate papers for science and arts majors) — and everyone takes politics.

Other key universities might be content with some twenty marks less. That the dividing line is so thin shows how fierce the competition is at the top. Marks count for everything as it is not possible to conduct interviews.

There is plenty of competition further down the education system too. Differences in success rates between different middle schools are huge: some "key" and university-attached senior middle schools send almost all of their students to university while another senior middle school just down the road may be able to manage only 1 or 2 per cent.

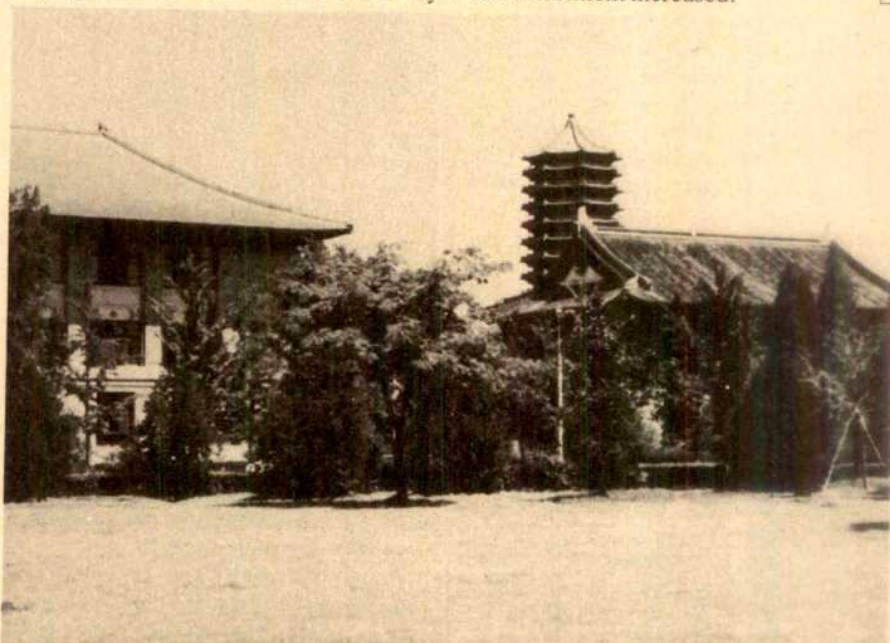
As one father with a daughter studying for university put it "it would be hopeless to try to enter university from a non-key school". Getting into a key school is again largely a matter of passing exams, although "influence helps"; competition at 12–13 years is tough, with parents busy helping with homework until ten every

evening. There are even "key" primary schools with preferred entry to key middle schools (although steps are being taken to do away with them). All this hectic studying will be familiar to the Japanese, as will another new development, the appearance of after-hours cramming schools. More than a few schoolteachers now run special private classes for those anxious to succeed.

The present system is a far cry from the Gang of Four days when the "soldier-worker-peasant" students were sent to university on the recommendation of their workmates; in those days there were no examinations (and nor were there degrees or graduate students).

There is still a small percentage of students who do not have to go through the entrance examination. At Qinghua, for example, around five per cent are instead taken in on the direct recommendation of their schools. Recommendations are taken only from a few privileged "key" schools that have built up close links with the university and have a tradition of excellence. Students will need to have the school president certify that their ability, character and health are truly exceptional before they have a chance.

Under consideration is the highly controversial suggestion that entrance requirements should be slightly eased for those that are willing to pay university fees. Indeed, it is even possible that most students will be asked to pay their way through university. It is for the best, in the long run, some say. The logic — pragmatic enough — is that for every student who pays, an extra student can be taken on and total enrolment increased. □



One of the best of China's educational institutions — Beijing University. Situated in the northwest of the city, most of the university's buildings are in the traditional Chinese style. The pagoda in the background is actually a water tower supplying the campus.

Intellectuals

Jam tomorrow — perhaps

YOUNG lecturers now entering the universities face considerable frustration and, like all intellectuals, have to cope on salaries well below those of industrial workers.

All university promotion was frozen several years ago while the government tried to sort out a new personnel policy. On top of that, retirements are not taking place as planned. In theory, staff should retire at 60 (if male) or at 55 (if female). Associate professors may continue until 65, and professors to 70, but they are not supposed to have administrative responsibility. However, these rules are hardly ever enforced. The majority of full professors are in their seventies or eighties and associate professors in their fifties or sixties. Perhaps the example recently set at the Party Congress, where the older generation retired from high office *en masse*, will have an effect!

Many argue that the older scientists have to stay on because the generation that should replace them missed their education as a result of the Cultural Revolution. But there seems to be no shortage of younger talent: there are many in their forties waiting for promotion and some have talented postdoctoral researchers in "cold storage" on scholarships overseas.

Whatever happens, a young lecturer is

certainly not going to live in luxury. Wages are only around 90 yuan a month. To put that in perspective, the national average salary is now 95 yuan a month, so a university lecturer earns much less than a factory worker. Top professors can earn 250 yuan a month, and university presidents as much as 330 yuan a month. But recently, much to the envy of city dwellers, peasants near big cities who have been selling their wares in city free markets have been reaching incomes of 550 yuan a month!

Indeed, the new rich can be spotted in the Shanghai suburbs, driving into town in the morning in privately owned trucks (which bear distinctive red number plates) loaded with vegetables and heading in the opposite direction later in the day loaded with cement. The neat little two-storey concrete houses they build now dot the countryside surrounding Shanghai.

Intellectuals will have to wait some while for similar luxuries. Although accommodation is provided at very low rates, there seems little sign of any pay rises on the horizon. No concessions were given to intellectuals in the current five-year plan and the word is that they will have to wait for their reward until the next. □

On your bikes to Qinghua

REMEMBERING where you parked your bicycle can be something of a problem on Qinghua University's vast campus; there are ten thousand students and rows of identical-looking bicycles parked everywhere. Qinghua is the top "key" university for engineering (with different engineering departments covering every speciality) and took the Soviet technical university as its model. Its main building, seen here, is in the Soviet monumental style but elsewhere there are reminders of the university's origins. Traditional one-storey buildings with upswept roof-corners, vermilion pillars and classical rock gardens remain on one part of the campus, while a lecture hall with a domed roof and a Greek portico recalls the pre-revolutionary days when the university was a preparatory school for those going to study in the United States.

Also a surprise is that there are several factories on campus — the university even runs its own 2,000 kW power station. The factories are there for three reasons: to provide practical engineering training for students, to carry out developmental work, and to produce experimental equipment and manufacture goods for the market. Qinghua is successfully making a small computer, three types of micro-computer, machine tools, electronic components and precision tools. Research is often supported through contracts with in-

dustry, around 90 per cent of the university's research funds are gained in this way.

One of the university's top priorities is to improve the standard of teaching. Too many staff are poorly trained (blamed on the turmoil caused by the Cultural Revolution) and classes crowded, with lectures



Qinghua's parking problem outside the main university building.

attended by 100–200 students and seminars by 25–30 students. But noticeboards advertising lectures by visiting foreigners show there is considerable fresh input. □

Biotechnology

Planning for a model institute

CHINA's first biotechnology centre is just a scale model at present, but within two years everything from research laboratories (including four P3 labs) and animal rooms to a library and accommodation for visiting scientists should be complete. Some 57 million yuan have been set aside for construction and US\$5 million for buying modern instruments and reagents from abroad. Shanghai, where the institute is to be, has thus stolen a march on Beijing where plans for a biotechnology research institute are still at the discussion stage.

Shanghai has capitalized on its rich biological research community in going ahead with the centre: most of the 130 young research staff will come from the large Academy institutes of biochemistry, cell biology, physiology, medical research and the like. But capital to establish the institute comes from the central funds of the State Planning Commission.

Much of the centre's research will be in "traditional" fermentation according to its vice-director Sun Yu-kun, who came from the Institute of Biochemistry. A conservative approach is being taken because capital is scarce and the institute must make money soon; genetic engineering has a long way to go before it turns a profit and can thus occupy only a small part of the centre's activities. Nor is there significant experience in "downstream" processing in China; first attempts are likely to be the production of small peptide hormones in yeasts. Growth hormone, which can stimulate milk yields, is a likely candidate as its chemical synthesis has already been achieved in China.

A more conventional project being given thought is to use immobilized enzymes to turn the galactose in milk into glucose and thus increase its nutritional value. Others are the production of vitamin C through fermentation, a process developed in Beijing's Institute of Microbiology; and the production of penicillin acylase — used in the manufacture of semisynthetic penicillins — by a method developed at the Shanghai Institute of Materia Medica. Wherever possible it seems that techniques already mastered in China will be built upon. But these are early days and it is not yet clear exactly what line will be pursued.

Links with foreign companies are also likely and the centre is close to a contract with a European company to produce both monoclonal and polyclonal antibodies as diagnostic kits; no such commercial kits are yet produced in China. Foreign staff are also to be invited to the institute, along with guest researchers from other parts of China. But Professor Sun seems a little pessimistic about further

prospects for joint ventures; foreign companies often just want to sell their product or want too much money for their patents — even though they are interested enough in the China market.

In the longer term, there are hopes for a hepatitis B vaccine. With 10 per cent of the population carriers the need is obvious. But there is much work to be done before there is any hope of mass immunization for costs are still far too high.

Shanghai Institute of Biochemistry

The molecular biology revolution

SHANGHAI'S Institute of Biochemistry gained world-wide renown in 1965 when it brought off the complete chemical synthesis of insulin — a technical *tour de force* requiring a seven-year team effort. After insulin came the chemical syntheses of vasopressin, oxytocin, angiotensin and luteinizing hormone releasing hormone (LRH); and then in 1981 another technical triumph with the total synthesis of yeast alanine transfer RNA.

But now the institute's interests are turning more towards molecular biology. Indeed, this would have happened a lot earlier if it had not been for the Cultural Revolution. During that period the institute's fame enabled it to continue in operation; but only to perform the same kind of research. In the world outside molecular biology was blossoming but there was no chance of absorbing "foreign" learning.

Professor Zhang You-shang, who now heads the institute, and his colleagues are well aware that the real value of chemical synthesis is in structure-function studies; during the Cultural Revolution they had to treat synthesis as an object in its own right.

Rapid progress is now being made in

A nationwide plan is also being worked out for biotechnology by the State Science and Technology Commission and considerable stress is expected to fall on applications in agriculture and aquaculture. But Beijing's plans to set up several more biotechnology centres may fall flat for want of trained staff. Professor Sun, at least, believes it better to steadily expand one institute than waste resources on competing institutes. □

molecular biology. A sub-type of the hepatitis B virus (not the one found in the West) has been cloned and sequenced: the surface antigen was subsequently cloned in both *Escherichia coli* and yeast, but in neither case was expression sufficient for use in vaccine manufacture; vaccinia virus is looking more hopeful with clinical trials perhaps a year or two away. Numerous other projects are under way.

Problems of a different kind also much occupy the mind of the director who is in the midst of reconstructing the institute in line with the new reforms. What should emerge is a slimmed-down and perhaps stronger research institute with a brand-new molecular biology laboratory.

First, overstaffing has had to be tackled. During the Cultural Revolution, the institute grew larger and larger, with even unqualified senior middle school graduates sent to do research. There was no incentive to keep staff numbers down for funds were allocated simply according to the number of people employed.

Now that there is a financial squeeze on the way, the director is having to exercise his new powers of control over institute affairs. The institute has been divided into 39 research groups, with their leaders

appointed by the director. This did away with one level of hierarchy — the division — which previously isolated research groups. And the number of researchers in each group is now strictly limited. Researchers are appointed by group leaders on three-year contracts. But what is to happen to the excess researchers? If the new reform documents were interpreted literally, they could be sacked. But that is actually out of the question; a little persuasion has to be used to make them move elsewhere.

In essence, what is happening here and in other institutes is that those who are not



The legacy of the Cultural Revolution adds to Professor Zhang You-shang's staffing problems.

chosen to join in a research team receive neither space, equipment nor research funds although they will still be entitled to their salaries. Such "people to be appointed" as they are called, are given every help to find work elsewhere. But they will not be sacked.

Special difficulties come with some of those sent to the institute during the Cultural Revolution on the recommendation of peasants, workers and soldiers but who do not have formal qualifications. Some have proved successful but as Professor Zhang put it "some are not so suit-

THE meeting hall of the Chinese Academy of Medical Sciences is this magnificent old building just a stone's throw from the "forbidden city". The Academy's headquarters are just across the road, alongside the Capital Medical College which provided the base from which the Academy sprang. The hospital itself was founded back in 1921 and has been through a number of name changes according to the political climate — during the Cultural Revolution it had a brief spell as the "Anti-US Imperialism Medical University". The Academy now runs 19 research institutes, most of them in Beijing with two of the biggest, Clinical Medicine and Basic Medicine, on the central site. Nine thousand staff are employed with 400 at the professor and associate professor level. Strongest research areas are in cancer, particularly epidemiology and prevention, and cardiovascular diseases. But major weaknesses remain in basic medical research and biomedical engineering. □



able, but it is not so easy to transfer them now, they are not so young. If they transfer elsewhere they will have to start again at the beginning". Such workers are likely to stay on for a while doing various temporary jobs. What kind of personal strains these readjustments are causing — and they come on top of personal animosities that must have developed during the Cultural Revolution — Professor Zhang did not say, but one sensed that the burden of his task is not light.

There is good news for the institute too, though, in that it is to have a new Laboratory of Molecular Biology, funded by the government and the Chinese Academy of

Sciences, with the aim of providing facilities every bit as good as in Western countries. Indeed the laboratory's budget is to be as large as that of the parent institute itself. If anything, it is to be modelled on Cambridge University's laboratory of the same name, the permanent staff is to be small and visiting scientists, including those from abroad, are to be encouraged to come on contracts of 1–3 years. Three floors of the institute will be given up to the new laboratory; some of the changes needed to set it up are very simple: just installing air conditioning will make it possible to do research all year instead of having to stop in summer. □

Cancer epidemiology

Mapping the mortality risks

EVEN if China is a developing country, the major causes of death follow patterns very similar to those of the developed countries. So cancer is a major concern and, with hepatitis and birth control, is one of the three priority research areas earmarked by the Ministry of Health. Epidemiological research is given particular attention, partly because epidemiology leads the way towards prevention and partly because of the publication in 1979 of the *Atlas of Cancer Mortality in the People's Republic of China* (China Map Press), which revealed striking patterns of incidence of the different cancers across the country.

The atlas is an organizational *tour de force*. The survey on which it is based, carried out between 1973–75, covered 96.7 per cent of the population (approximately 800 million people). Ironically, the dispersal of many professional people to the countryside by the Cultural Revolution probably helped to make the compilation of the atlas possible and so may represent one positive outcome of this unhappy time.

Although the total cancer mortality (approximately 25 per cent of deaths) is similar to that in developed countries, the atlas shows that the overall frequency of particular cancers differs markedly from those common in the West. Stomach cancer is the most common form for both men and women in China. For men, oesophagus, liver and lung cancer follow in that order of frequency, while for women the order is cervix, oesophagus and liver. In Britain, for example, lung cancer is the most common among males followed by colorectal, stomach and prostate cancer. For women, breast cancer is followed by lung and colorectal cancer.

That environmental factors may be important is suggested by the marked variation of frequency in different parts of the country. Overall rates of death from cancer are highest in the south-east coastal provinces, in Shanxi in central China and in parts of the border regions with Mongolia. Nasopharyngeal cancer shows a strik-

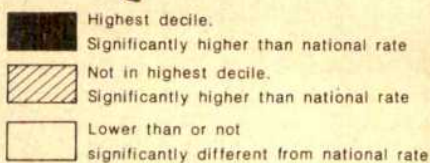
ing focus in Guangdong and Guangxi provinces in the south where it is almost as common as stomach cancer, whereas in Xinjiang Uygur in the far northwest, it is rare. Cervical cancer shows very high rates in Shanxi and inner Mongolia. Liver cancer (see map) is highest in Guangxi and along the south-east coast — interestingly, across the water in Japan these cancers are also a major concern. Other notable features include cancer of the penis, admittedly rare, which occurs to a large extent in the areas of highest incidence of cervical cancer.

This huge database, together with accumulating knowledge of known or suspected cofactors — hepatitis B virus (HBV) in liver cancer, Epstein-Barr virus in nasopharyngeal cancer and smoking in lung cancer — have fuelled a search for other factors which may be important.

Sun Tsung-tang at the Cancer Institute, Chinese Academy of Medical Sciences, Beijing, is investigating the possible role of aflatoxins, the fungally-derived contamination of cereal foods. In Qidong county, Jiangsu, high levels of aflatoxin contamination of foodstuffs have been found. Liver cancer rates are also six times those in Beijing, but the frequency of HBV carriers is only 2.5 times as much. Sun plans a four-year study which aims to reduce dietary exposure by screening foodstuffs with monoclonal antibodies against aflatoxins and by chromatographic techniques he has developed.

The role of aflatoxins is also being investigated by the Shanghai Cancer Institute on Chongming island, in the Changjiang (Yangtze) delta, again a region with high rates of liver cancer. In a longitudinal study of 40,000 people, details of diet are being combined with an extensive investigation of aflatoxin contamination.

Shanghai has had a cancer registry since 1963. The city has very high rates of stomach, liver and oesophageal cancer in both sexes, but, most notably, very high rates of female lung cancer. About 50 per cent of Shanghai males smoke cigarettes and the attributable risk of smoking for



Age-adjusted male liver cancer mortality for 1973–75 in relation to the national rate.

lung cancer is 80.5 per cent. On the other hand, only six per cent of Shanghai women smoke, and the corresponding association is as low as 19.3 per cent. With poor ventilation and traditional practice, there is a possibility that cooking oil fumes may account for some of the high female incidence. Gao Yutang of the Shanghai Cancer Institute is carrying out laboratory and epidemiological studies; a causal study of 220,000 adults was begun in 1983 in collaboration with the US National Cancer Institute.

Dietary factors have also been implicated in stomach cancer. Zhang Rufu of the Beijing Institute for Cancer Research has analysed gastric juice from a total of more than 40,000 people in high-risk regions such as Gansu province in the north-west and Fujian in the south-east as well as in low risk regions elsewhere. High risk is associated with high nitrite levels and the fungus *Aspergillus versicolor* in the stomach. Atrophic gastritis is also more prevalent in these areas, possibly as a result of poor diet which, at the end of winter, can be dependent to a large degree in some areas on contaminated pickled vegetables.

It is too early to have a clear picture of the role of dietary and other environmental factors in the development of cancer, but given the heterogeneity of diet and surroundings in China and the heterogeneity of cancer incidence shown by the atlas, much may yet be revealed.

Nigel Williams

Polytechnic education

University as industrial partner

THE Beijing Polytechnic University (BPU) thinks of itself as China's version of the Massachusetts Institute of Technology (MIT), perhaps not now but sometime. It has a number of competitors, most obviously the Chinese Academy's own University of Science and Technology, transplanted south from Beijing to Hefei during the Cultural Revolution. In the long run, BPU's advantage may be its location in Beijing, which is where most people seem to want to live.

The university lives on a sparkling new campus in an industrial suburb of the capital. As in much of the rest of Beijing, buildings wreathed in scaffolding are a sign of things to come. The university's president, Professor Fan Gongxiu, is able to boast of a close relationship with the municipality of Beijing that should keep his institution in the forefront of the city's expansion for some time to come. And as at MIT, there are more tangible achievements to brag about — a state prize for the development of a laser printer (now being manufactured at Barbin, in the north-east) for example, and the second prize in the national Go competition. (Go is the



Dong Zhichang (right) and Zhang Zhi of BPU's Chemistry and Environment Engineering Department won a Beijing Science and Technology Achievements award for developing a crystal glaze.

board game sometimes counted as the Chinese equivalent of chess.)

By some other standards, the university is small. Student enrolment is 3,800 (of whom 300 are graduate students), but is expected to reach 4,500 by 1990. Usually, BPU sets out to recruit students who have already started work in industry, some 500 of them this year. As at other Chinese universities, the ratio of students to staff is luxurious (there are 1,250 members of the teaching staff, 750 of whom are professors or lecturers, and a roughly equal number engaged on other duties); the explanation is that a student's enrolment entails total responsibility; students must not be merely taught but housed, supervised and even instructed in what is called "political science".

Stimulated by an experiment in educational reform launched in 1983 by the municipality of Beijing, the university has paid special attention to "moral education", which takes the practical form of

engaging students on social work among old people and the like.

The most striking sign of what the university does for industry is the source of its funds; this year, 8 million yuan will come from the central government but 6 million yuan from industry. On top of that, the university has this year brought in 4 million yuan for research, chiefly as research contracts with industrial enterprises.

The demand for the university's services as an industrial partner has meant that 20 substantial projects have been turned away this year. Selfconsciously, the university reckons to be able to choose the projects with the "best economic value", but a collaborative research project will not be undertaken unless there is a suitably qualified member of the faculty who is, moreover, willing to take on the responsibility.

So what impression of the quality of industrial research in China has this well-placed university formed? Professor Fan acknowledges that many (but not all) industrial enterprises are much in need of skilled technical people, but he does not agree that industry needs always to be capable of carrying out its own research and development. That may be the pattern in the West, but in China it may make sense, and economize in scarce resources, that institutions like BPU should take on central research and development for a group of industries.

Often, it seems, the intimacy of the relationship threatens to turn the university into an industry in its own right. In 1981, Fan says, the industrial design department at the university embarked on a study of single-board microprocessors, from which sprang no fewer than a score of novel designs. So now the university has a factory with 100 workers which, last year, earned 20 million yuan from the sale of its products. Among other things, Fan says, the factory is a model for other factories elsewhere in China.

The curriculum at BPU is rigorous, perhaps even more so than at MIT. The standard course lasts for five years, beginning with basic science instruction. There is a language laboratory to ensure that everybody has a chance to learn some language other than Chinese, and facilities for art instruction and practice. Project work plays an important part in the curriculum in the later years of the course, and BPU reckons to do exceptionally well in the national competitions regularly organized in China for those with engineering innovations to show off.

For a university as new as this (BPU came into being only in 1960, directly under the wing of the municipality, and most of its work was suspended during the Cultural Revolution), international relationships are prominent and strong, espe-

Fighting restrictions



LIANG Zhiquan (left) and Shen Yufei in the Department of Molecular Biology and Biochemistry at the Institute of Basic Medical Sciences (Chinese Academy of Medical Sciences), Beijing. Founded in 1919 as part of the Beijing Union Medical College, it now has a scientific staff of around 370 and the largest department is that of molecular biology.

The emphasis is basic work on well-defined Chinese medical problems. As in Shanghai (see page 217), a hepatitis B virus subtype has been cloned and sequenced — an essential step towards the production of a recombinant vaccine. The use of gene probes to study and detect prenatally α - and β -thalassaemias, which are quite common in Guaxi, Guangdong and Sichuan, is being developed by Wu Guanyu in collaboration with other institutes in the affected regions. Successful prenatal diagnosis of α -thalassaemia has been carried out.

A new and important role for the institute is the development of restriction enzyme production and Qiang Boqin is keen to hasten the time when China can be self-sufficient in these reagents.

Progress at the institute has somewhat overwhelmed the old buildings, but hopes are high that brighter more spacious accommodation will be available in a new building within four years.

Nigel Williams

cially with institutions such as the State University of New York at Buffalo, Northeastern University and the like. One objective is to let potential members of the Beijing faculty complete their studies in a stimulating environment, another to add to the university's competence in industrial research.

Among the young people of Beijing, BPU is clearly a favoured institution. In the annual competition for university entrance, some 7,500 students a year, or roughly ten times as many as the annual entry, put BPU first on their list of desirable universities. So why not grow faster? Fan's reply contains an element of paradox: even an industrially oriented university should not grow too quickly for the health and wellbeing of China's industry. But, secretly, the objective may be to avoid jeopardizing quality in the chase for numbers. □

Plant taxonomy

South China's germ-plasm tank

TRANSPLANT the Royal Botanic Gardens, Kew, from the suburbs of London to a subtropical climate and you would have the South China Institute of Botany, twenty tortuous kilometres from downtown Guangzhou, the provincial capital of Guangdong. But the institute has long since outgrown its role as the taxonomic centre of luxuriant South China, with interests that range from plant ecology to agricultural botany.

The origins of the institute parallel in interesting ways the emergence of modern China from its feudal past. Guangdong was the base from which, in the 1920s, the revolt against the surviving feudal barons was organized by Dr Sun Yat Sen (after whom the university of Guangzhou is named). The province then had the sense of being at the centre of reform in China. For a time, in the 1920s, an island in the Pearl River was the site of the military academy whose students at various times counted among their number not only Mao Tse Tung and Chiang Kai-shek (later the Kuomintang leader) but also Zhou Enlai, the Chinese Prime Minister in the period immediately after the Cultural Revolution.

By 1928, the plant taxonomist Chung, then based at the Sun Yat Sen University, embarked on an ambitious taxonomic study of South China. Over the succeeding twenty years, undisturbed by revolution, invasion (by the Japanese) and civil war, Chung and his team, which never exceeded 26 people, scoured the region for plant specimens, which now make up the core of the institute's herbarium, a vast collection of 600,000 dried specimens. Soon, the institute hopes, the herbarium catalogue will be computerized. And there are plans to transfer the whole of the herbarium to an air-conditioned building.

The present director, Dr Kuo Chun-Yen, has his intellectual roots in the Middle West of the United States; a graduate of the University of Shanghai, he took himself to the University of Chicago for graduate studies in 1947 and was then stranded at the University of Michigan (at East Lansing) because the United States and the new Chinese People's Republic did not establish diplomatic relations with each other until 1955. The result is that Kao bubbles with enthusiasm for the plant-breeding unit that covered the Middle West with hybrid corn in the 1960s. In September this year, he was packing his bags for yet another visit to East Lansing, an extended stay supported by the US National Academy of Sciences.

Kao's interests in agricultural botany reflect the present pattern of the institute's work. The establishment was moved from the university to its present site in 1954, and four years later spawned

the adjacent 30 hectare botanic garden. The subtropical climate has ensured that, in a mere thirty years, traces of the previous army barracks have been obliterated by lush green vegetation. In 1955, the institute also acquired the 1,200 hectare site in the mountains southwest of Guangzhou which has become an arboretum of such distinction that it is likely to be the centre of one of UNESCO's Man and Biosphere projects.

Nationally, the South China Institute is one of three laboratories whose interests between them span the whole taxonomy of Chinese plants (the others are at Shanghai and Beijing). Jointly, the three institutes have embarked on the compilation of a comprehensive Chinese flora, a gigantic undertaking whose timescale remains undefined. But in practice, the institute's interests inevitably centre on the agricultural needs of South China. The techniques of meristem culture have become the spearhead of plant breeding, here as elsewhere in China. Kao is especially pleased with a project, now in the development stage, to propagate a species of begonia growing naturally on rocky surfaces which has been collected almost to extinction on account of its medicinal properties (but which, in any case, makes a pleasing tea-like beverage). Kao emphasizes that the topography of much of China is unsuitable for the growth of paddy rice, for which

reason there is a great need for the development of other kinds of crops. Kao figuratively snaps his fingers with frustration that the Chinese gooseberry has been transmogrified into New Zealand's kiwi fruit. The institute is engaged in the general concern to develop crops that may be grown successfully on the island of Hainan, the strategically important island off the south coast of Guangdong that faces North Vietnam, as well as in what seems to be the national concern that lychee fruits should be prevented from deteriorating during their distribution.

By most yardsticks, the South China Institute is a huge laboratory, with a staff of 560, of whom 260 are scientists with degrees. It is as well equipped as anybody could ask. As at most other laboratories in China, there is a curious contrast between the internal finish of laboratory buildings, which seem unusually to be shabby sources of powdered cement dust, and the spick and span appearance of the instruments, mostly newly imported from overseas.

Like the other major institutes of the Chinese Academy of Sciences, the South China institute is an important centre for the recruitment and training of professional scientists. The competition for a place at the institute is keen, not least because it seems to be generously provided with funds to support travel overseas. For the past six years, the institute has been regularly sending people to work overseas not merely so as to read for PhD degrees at universities in the West but also

Fudan powers nuclear industry

STANDING around president Xie Xide in the photograph below are the senior science staff of Fudan University, one of China's top comprehensive universities. In contrast to nearby Japan, women professors in science faculties are not uncommon.

Fudan University was set up in Shanghai in 1952 in the days when a major first task of staff was to translate Soviet textbooks. Nowadays, most staff have been to study in the United States or Europe and the university is adapting to the new open policies. A new School of Technological Sciences

was set up last year, not to train engineers, but to produce top graduates with the scientific grounding necessary for research and development. Among research in progress is work on oil prospecting software for a homebuilt high-speed computer, zeolite catalysis, control of pests by viruses, and VLSI (very large scale integrated circuit) photoresists. There is also one of the nation's few departments of nuclear science, some of whose graduates will go to the new nuclear power plants now being constructed.

The teaching system is also becoming more liberal with students allowed to choose courses to go with their majors and the introduction of a US-style credit system. There is talk — as in Qinghua University — of an exam at the end of the first year to weed out students who are not making the grade, although it is bound to face opposition. Like universities elsewhere the staff is divided into those who do research, and those who mainly teach with perhaps a third of their time free to research. Rotation between groups takes place, but irregularly. Research facilities have much improved in the past few years with a massive infusion of new imported equipment into the science building.



Left to right: Hua Zhong-yi, vice-president; Zheng Qi-ke, nuclear science; Wang Li-hui, materials science; Xie Xide, president; Wu Li-de, computer science; Su De-ming, biology department; Zhou Dun-ren, foreign affairs; and Gao Zi, chemistry department and recently at Imperial College, London.

to work on collaborative projects. As part of a collaboration with the University of Utah on photosynthesis under the auspices of the Carnegie Institute of Washington, for example, Kao has two of his people at Salt Lake City on a five-year stint.

Reform has also begun to make its mark. The research programme at the institute is being formally evaluated by a committee including people from outside the institute, and a process has been set up for assessing the performance of individual researchers so that, over the years, people can be persuaded that their personal interests coincide with the national interest that the scientific labour force should be more mobile.

As elsewhere, the Cultural Revolution is philosophically regarded as a phenomenon belonging to the past. Kao and many of his staff were sent off to the countryside at the beginning of the upheaval, but he says that after a little while, they were able to move some of their equipment into exile as well, and get on with their work. During the period, they were able to produce three volumes of advice for barefoot doctors on the medicinal use of Chinese plants, as well as giving some of the peasants with whom they worked first-hand instruction on the use of new techniques and novel cultivars. And even now, on a strictly voluntary basis, the laboratory thinks it worthwhile that some of its scientists should work closely with peasants in the countryside, providing some of the services of what would elsewhere be known as agricultural extension services.

Guangzhou's botanic garden is the creation of an Edinburgh-trained agronomist, Professor Chen Fung Huai, now 85 and living in retirement in the grounds of the South China Institute. Chen is now a frail shadow of the man who, in the period after 1949, seems to have made it his personal mission to scatter botanic gardens about China.

Botanic gardens, he says, are not parks or playgrounds, but "open-air laboratories". Each new site is a different challenge. People have to worry not simply about the climate but the pH of the soil, and must be able to guess how the microclimate of each part of a new garden will change as it matures. During his career, Chen has founded botanic gardens in places such as Beijing and Nankin.

Chen is now the South China Institute's most distinguished resident. Kao, the director, says that he has to take care to preserve Chen's energy, protecting him from visitors whenever possible. It is one mark of the way in which ties between China and the rest of the world have survived the upheavals of the past half-century that it should be possible to have a polite conversation about the temper of a Scottish university in the 1930s in such a faraway place as this suburb of Guangzhou. □

Guangdong

The centre is a long way away

SOUTH China is as different from North China as is, say, Louisiana from Massachusetts. The topography, to be sure, may look much the same, but heat and humidity give both southern regions a distinctive sense of being different. But there is another influence in South China — Hong Kong and, to a lesser extent, Macao. The free port ("special economic zone") surrounding what are called, in Hong Kong, the New Territories is the most successful of the four now operating, and has acted like a magnet in shaping the direction of economic activity in the province of Guangdong. One curious sign of the proximity of Hong Kong (about 100 km away) is that it has been for the past year permissible for people in the province to watch Hong Kong television programmes (for which purpose an imported receiver is required). There can hardly be any greater contrast than that between the staid Chinese channels, which broadcast Party meetings, accounts of industrial improvement and scenes from well-ordered family life, and the steady output of violence by video that distinguishes the Hong Kong stations.

Guangzhou, the provincial capital, is also the seat of a branch of the Chinese Academy of Sciences, a distinction that is somewhere in between an administrative decentralizing step and a means of recognizing the importance of a provincial centre. But the Guangzhou branch differs from that in, say, Shanghai in that its relationship with the provincial government is close. Formally, there is a Guangdong Academy of Sciences which is directly responsible for the seven provincially supported research institutes, and which manages the local branch of the national academy as well.



With travel restrictions inside China eased, the Great Wall is always full of tourists from all over the country.

Thus the local branch is a kind of Cantonese contract manager of the six institutes of the Chinese Academy of Sciences in the locality (oceanography, geology, chemistry, electronic technology and energy conversion as well as the South China Institute of Botany — see opposite). The clutch of institutes financed by the provincial government, in fields such as geography and microbiology, serve largely provincial needs; officials are proud that, in the past few years, the attention the microbiologists have paid to the improvement of mushroom strains has, for example, made it possible to increase the income of peasant mushroom growers by 10 million yuan a year. Another proof of Guangdong's capacity to look after itself is that the Academy seems to have been able to open a new research institute, that for electronic technology, at the height of the Cultural Revolution.

For all its operations, the Guangdong Academy gets 25 million yuan a year from the Beijing Academy and 7 million yuan from the provincial government. Officials of the local academy are enthusiastic supporters of the government's policy of modernization. They see it as one of their principal tasks to persuade more scientists into industrial employment, to which end they are doing everything they can to have their own institutes work more closely with industrial partners.

The Guangdong management is plainly not informed of every detail of what its institutes are up to in these fields, however — the central office had not learned in late September that its institute of electronic technology had sold a software package to a Hong Kong company for HK\$100,000. But that is entirely within the rules. Only if the earnings of individual institutes became a large proportion of their budgets would it be necessary to consider whether the income should be used to help finance other institutes.

Coping with growth may be Guangdong's only source of serious worry. For the time being, the officials say, there is enough money to keep the institutes hard at work. The supply of people is sufficient, but there is some concern about the quality of those leaving the universities. Equipment is no great problem now that the institutes with research reputations can get their hands on foreign exchange.

So should not something be done to improve the universities, and to increase their intake of students? Here again, Guangzhou echoes Beijing. For the time being, there can be no substantial increase of the university population. The immediate hope is in continuing education, either at the workplace or by private study, with help provided by educational television programmes. □

Shanghai biology

Largest city looks to itself

SHANGHAI, China's largest city (on the strength of its ports and industry), has every reason to regard Beijing, China's capital, as an administrative centre somewhere to the north. The relationship between the two cities resembles, for example, that between New York and Washington DC, or, in the nineteenth century, between Manchester (England) and metropolitan London. This is the spirit in which Shanghai takes pride in being one step ahead of the capital. Institutes of the Chinese Academy of Sciences at Shanghai have thus been first in the field with the synthesis of insulin, and have designed China's indigenous nuclear reactor.

Another sign of the way the wind is blowing is that the local branch of the Chinese Academy of Sciences is now busily welding together on a single campus a clutch of biological institutes that, between them, will have more than 3,000 qualified people on their books. The intellectual architect of this scheme is Professor Cao Tianqin, now the president of the Academy's Shanghai branch and director of its division of biological sciences.

Apart from the biochemistry institute (of which Cao was the director until a few years ago), there are institutes of physiology (housed in a Victorian gothic mansion), organic chemistry (oriented towards natural products of medicinal value), materia medica (more so), cell biology, brain research, plant physiology and entomology. The laboratories are united into a kind of common purpose, not the least by an ambition to be doing today what Beijing may do tomorrow.

The Shanghai institutes are especially proud of their overseas connections. Cao took his PhD at Cambridge. Professor Mei Zhen-Tong, the woman who is director of the Institute of Physiology, is similarly trained overseas. Professor Chang, who founded the brain research institute by fission from physiology in 1981, had returned from a distinguished career at the Rockefeller Institute, New York, and at John Hopkins. The Australian connection is also conspicuous at Shanghai, especially in plant physiology and entomology.

This complex of laboratories is splendidly equipped. During the past few years, there seems to have been very little restraint on the supply of hard currency for the purchase of equipment, so much so that there are occasional signs that some of what has been bought in from overseas has not yet been used for its intended purpose. One mark of the state of the equipment at the Shanghai institutes is that the important suppliers from the United States and Europe have thought it worthwhile setting up agencies in the city.

Although most people in these biological laboratories recognize that the future lies with molecular biology and its applica-

tions, for the time being there are two serious impediments. First, in spite of many efforts during the past few years, plans to provide a central agency for the supply of essential reagents such as restriction enzymes have not succeeded; the purity and activity of the indigenous products are variable, so that too many researchers and their helpers must spend precious time making their own. Much the same applies to the supply of radioactive chemicals. The specific activity of reagents labelled with active carbon is never great enough to satisfy the molecular biologists.

Meanwhile, there is no shortage of interesting work on hand. The organic chemistry institute, with a total of 1,200 members of staff (500 or so employed on pilot-plant work) spread their interests from the role of nickel and molybdenum in catalysis to the search for medicines. One such success is the characterization and purification of an abortifacient in a traditional medicine which consists of a polypeptide with 230 amino acids. In the pure form, microgram quantities of the material appear to be effective. The laboratory itself is manufacturing the material for distribution in China, and estimates that more than one million women have so far

Acupuncture boom punctured

ACUPUNCTURE is still widely used in China for the treatment of various ailments, rheumatism, for example. But there now seems to be a retreat from the assertion, common during the Cultural Revolution and believed by many Western physicians at the time, that acupuncture can be an effective analgesic in surgical operations.

Dr Jiang Zhen-yu (C.Y. Chiang), from the Shanghai Institute of Physiology, is judiciously cautious on the present use of acupuncture. He says that the ministry of health has put out a decree restricting the analgesic use of acupuncture to operations on the thyroid, the diaphragm and similar procedures. The general use of anaesthetic acupuncture has otherwise fallen into disuse. And there is no means of telling how widely practised are the uses allowed by the government decree.

Jiang insists that the new scepticism does not extend to the therapeutic use of acupuncture. In saying that, he reflects one of the most striking features of Chinese health care to visitors from abroad: the apparently easy coexistence of scientific and traditional medicine. The explanation, to scientist apologists, is that traditional medicine will be found to be just as objective when biochemists have identified the active principles of traditional remedies, dried bears' gall-bladder (a treatment for kidney stones), for example. □

made use of it. The obvious next step is to tell which parts of the molecule are essential for the biological activity in the hope that synthetic production will be possible. Similarly, there is a continuing hunt for antimalarials; the active principle of the *Qinghan* species, identified at the laboratory in 1979, has now been synthesized.

Between them, the Shanghai biology and biochemistry laboratories span a vast range of interests, from neurophysiology and the search for new drugs to classical environmental physiology.

The laboratories also have a crucial educational role. The organic chemistry institute alone has 150 graduate students, and is now selecting 6 or 7 MSc graduates a year for its PhD programme. With the help of imported computer equipment, the laboratory is also setting itself up as a national centre for chemical data.

Most people in this Shanghai complex think they will do well out of the reform. The good basic research people, they say, will succeed in the competition for research grants from the National Science Foundation, to come into being next year, while those whose work has practical applications (the cell biology institute, for example, is strong on the endocrinology of fish reproduction) will also thrive.

The cell biology institute hopes, under the reform, to become an "open laboratory", a distinction that would allow it to take in researchers temporarily from university departments, to be more free than at present to spend funds on overseas travel and on inviting visitors from overseas. (Already it has a suite of laboratories, paid for by the West German Humboldt and Thuyssen foundations, which houses two West German doctoral scientists in spectacular luxury by Chinese standards.) But greater freedom in the selection of graduate students is ultimately the prize.

Under the new system, will it be possible to safeguard the interests of basic research? Most of the laboratory managers are conscious of the need, even when they add that China's industry is too short of skilled help. The most obvious danger is that even when people succeed in the competition for research grants from the National Science Foundation, their salaries will remain what they are at present, while there is at least a chance that colleagues who work on industrial contracts will find that their personal rewards are substantially greater than their salaries. Given the resentment about the poor rewards of research now building up in the laboratories, laboratory managers will be tempted to share outside income on some egalitarian basis. But even so, laboratories concerned primarily with basic research will be at a disadvantage. Nevertheless, the president of the Academy of Sciences, Professor Lu Jiaxi, says in Beijing that "they'll have to wait another five years" (until the next five-year plan) to share in China's growing prosperity. □

High-energy physics

Towards home-grown mesons

WHY should a country such as China be building a particle accelerator when countries such as Britain wonder whether they can continue to belong to international organizations such as CERN, the European high-energy physics laboratory at Geneva?

The answers are various. The technology of accelerating charged particles is valuable in itself. Worthwhile accomplishments in such a field are a necessary price to pay for collaboration with others. And in any case, as the economy of China grows to match those of the large industrial nations, it will be natural that China should be in high-energy physics.

But the clinching argument is probably similar to that which sustained high-energy physics in Britain and France in the 1960s; there is a group of Chinese physicists with ability and political influence who consider that China without an accelerator would be a contradiction in terms. Even though the ambitions of the period immediately after the Cultural Revolution have been scaled down, the government is generously backing construction of a 2 GeV electron-positron collider.

The result is the large elliptical hole in the ground now taking shape at the Institute of High-Energy Physics at Beijing. By 1988, this will have been equipped with the appropriate vacuum chamber and magnets, within which there will circulate two oppositely directed beams of electrons and positrons, each beam with an energy of 2.2 GeV.

In these terms, BEPC (for Beijing Electron-Positron Collider) will be most closely comparable to the electron-positron collider called SPEAR II, which operates at Stanford but the design group hopes to attain a luminosity (collisions per square centimetre per second in the colliding region) greater by at least an order of magnitude (specifically $1.7 \times 10^{30} \text{ cm}^{-2} \text{ s}^{-1}$).

This, of course, is both a far cry from the energy of machines such as LEP (being built at CERN), which should give electron and positron beams of 86 GeV with the possibility of an extension to 135 GeV per beam. It is also a far cry from the plans announced at the great national meeting on science and technology in November 1978, when the ambition was to build a machine for accelerating protons to 50 GeV (a more powerful version of CERN's super-synchrotron, say).

That was the goal as recently as four years ago, but detailed design work showed, according to vice-director Zhang Houying, that the cost had been underestimated by 1,000 million yuan or so. The machine now being built is a utility electron collider; the energy of each beam can be tuned between 2.2 and 2.8 GeV, but the option of extending the beam energy to 4.7 GeV has been dropped. To begin

with there will be only one point on the storage ring at which electron-positron collisions will be induced (with provision for a second colliding zone to be added later) and the storage ring will be equipped with an oscillating magnetic field to generate synchrotron radiation with usable flux in the keV range.

One relic of the heart-searching of the past few years is a 35 MeV linear accelerator for protons, built almost entirely from Chinese components, which is being ingeniously converted into a means of generating fast neutrons (for the treatment of cancer patients) and short-lived isotopes.

BEPC itself is the result of a government decision at the highest level (the



Beijing's storage ring takes shape.

State Council). The cost of the construction phase (240 million yuan) is being borne directly by the government even though the institute is an integral part of the Chinese Academy of Sciences' network. ("We couldn't afford it", said one Academy official.) According to vice-director Zhang, the eventual running cost of the new machine will be some 20 million yuan a year; it is still not clear which part of the government will meet the cost, but the Academy seems determined that it should be the government direct.

Since the ground-breaking ceremony in October 1984, work has pushed ahead quickly. The 201-metre tunnel for the injecting linear accelerator (which will produce alternately electrons and positrons at 1.1 GeV) is almost complete, 8 metres below ground level. Concrete is being poured for the containment walls of the tunnel to house the storage ring. Construction should be complete by the end of 1986, with operation 18 months later.

Intrinsic interest apart, BEPC is a marvellous illustration of the problems of building a big high-technology machine in China. Part of the objective has been to

demonstrate, for self-confidence if no other purpose, that the design can indeed be carried through; so the control system (which detects the positions of the electron and positron beams, adjusting the magnetic fields to keep the beams on course) has been elaborately designed around a network of novel Intel integrated circuits.

But as yet, the design is unfinished; last month, no fewer than 15 people from Beijing were working at the Stanford Linear Accelerator Center on the details of a design that will, with luck, incorporate the latest in accelerator technology.

The physical components of the accelerator are more teasing problems. According to Zhang, the sections of the aluminium vacuum chamber are being made in the United States because they are cheaper that way (\$50,000 against 700,000 yuan). The same applies to electronic components, which are 1,000 times as expensive in China as in the United States.

On the other hand, the klystrons that will generate the microwave power to accelerate electrons in the linear injector section are to be made in China because their export from the United States is forbidden; the high-energy physics institute has had to rely instead on the Ministry of Electronics to commission the manufacture of the 16 klystrons required for the linear accelerator (peak power output, 15–20 MW at 2.856 GHz), no doubt to the benefit of China's defence industries.

The US government has nevertheless relaxed the strategic embargo to allow the purchase of a VAX 11/830 computer after 18 months of negotiations, but in return for a promise that the machine will be used only for the collection and processing of data from the drift chamber detector being built for the sole experimental section of the storage ring.

What will the machine accomplish? Some physics, without doubt; even by 1988, the products of electron-positron collisions with total energy between 4 and 5 GeV will not fully have been worked out. There will be plenty of charmed mesons and the institute intends to concentrate on careful measurements of the properties of these particles and the details of the decay of the heavier analogue of the electron and muon (the tauon).

The more enduring objective is to show that China can build an accelerator, and in the process win access for Chinese physicists to more powerful machines being built elsewhere. Already there are members of the institute working at Brookhaven and the National Accelerator Laboratory (Fermilab) in the United States, as well as at Stanford and CERN. The institute also hopes that its new machine will become a focus for collaboration with other developing countries.

For reasons such as these, the institute seems not to be under serious pressure from the government to be more directly

involved with industrial development, some of which is in any case stimulated by the need to develop novel components for the accelerator. With more than 2,000 people (1,200 of them scientists and engineers), the institute hopes to have more than 100 graduate students in the year ahead and a quota of 10 postdoctoral fellowships a year, chiefly as a means of holding onto those returning from abroad.

Curiously, the institute seems to have been spared the worst excesses of the Cultural Revolution, although people like vice-director Zhang (an electrical engineer) spent much of the time as a member of a cadre work-school laying electric-

ity power lines. Its partial immunity seems to have stemmed from a Maoist dictum that it is important to "keep on dividing the constituents of matter" as well as from its antecedents; the institute is an offshoot of the Institute of Modern Physics established in Beijing in 1950, from which has also sprung the Institute of Atomic Energy (in the southwestern suburbs of Beijing) and, less directly, the Shanghai Institute of Nuclear Energy (responsible for the design of the 300 MW indigenous reactor) and the Lanzhou Institute of Modern Physics with a programme of low-energy nuclear physics. The Moscow Institute of Physical Problems is the closest analogue.

Space programme

Next, the non-stick heat pipe?

CHINA in space is not the joke suggested by its first satellite, launched on 24 April 1970, and still affectionately remembered for its broadcasts (audio, not video) of a folk song. Indeed, there are no doubt many who would consider that the rapid development of China's rocketry is almost the opposite of a joke.

On the civilian side, China has since 1970 launched a total of 16 satellites. Last year (8 April) the Ministry of Astronautics succeeded in putting a small satellite into a geosynchronous orbit, preparing the way for the series of telecommunications and broadcasting satellites scheduled for next year and thereafter.

Indeed, the ministry is now talking up the possibility of selling launching facilities, just as do the managers of the Ariane and US shuttle programmes. Zhang Jiqing, an engineer with the ministry and director-general of what is called the Foreign Affairs Bureau, says that there have been conversations about satellite launchings with the Netherlands, Sweden and even the British government (represented by the Rutherford Appleton Laboratory of the Science and Engineering Research Council, a grant-making agency). But nothing has yet been fixed.

The ministry says its interests are entirely civil, but it goes without saying that the civil space programme is an outgrowth of the military programme on whose behalf patches of the Pacific Ocean are from time to time declared out of bounds to shipping so that a military rocket may plump down without doing damage.

Indeed, Zhang's simple answer to the simple question of what a developing country such as China is doing in space is that the country has a need (for telecommunications and broadcasting satellites especially) and also the means. The liquid-fuelled rockets (using unsymmetrical dimethyl hydrazine as fuel, burned with liquid oxygen) have been labelled sequentially as "Longmarch" I, II and III, with IV and V under development. They have their roots in a series of developments thirty years ago, when the military rocket

programme was begun.

There is a host of research institutes catering for the needs of the military and civil space programmes, many of them the property of the Ministry of Astronautics, others operated by the Chinese Academy of Space Technology, which is itself influenced (and partly financed by) the National Commission for Science and Technology for National Defence, constitutionally similar to the civilian academy on which the Chinese Academy is dependent.

There are special research institutes in fields such as microelectronics, but the design and fabrication of launch vehicles is the responsibility of an industrial organization, the Wangyuan Industrial Corporation.

There is no reason to doubt what people such as Zhang say about the civilian character of his ministry, which seems to have been hewn out of the defence establishment, and given a separate identity, so as to allow military development to continue in isolation — and secrecy. In this diligent separation of the two faces of research,

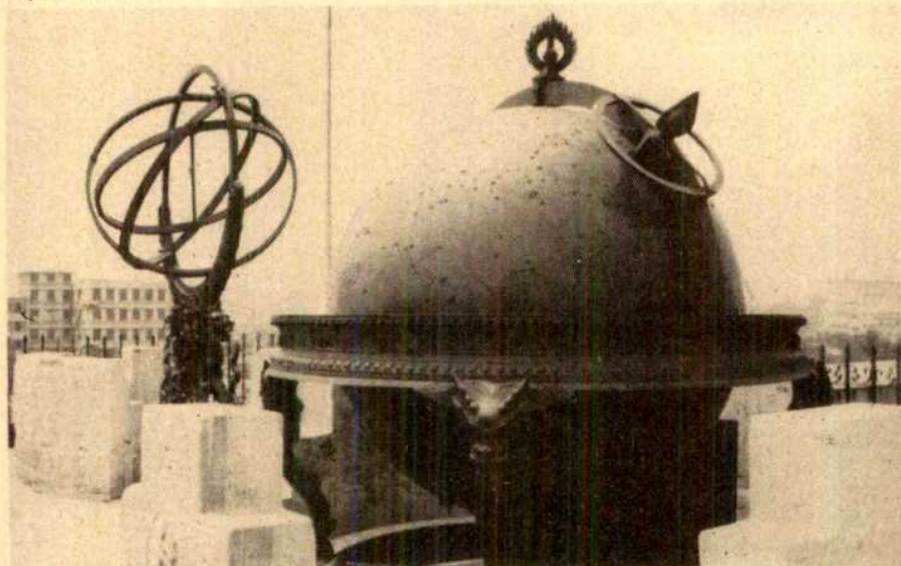
China has followed the Soviet Union in its organization.

But China's special claim on attention, according to Zhang Jiqing, is the development of techniques for recovering satellites from orbit (by means of a retrorocket and a parachute) and the use of a single rocket for launching several satellites. There is nothing remarkable about the sequence in which the rockets at the ministry's disposal have been developed. The first was a three-stage affair, with two liquid-fuel stages and a solid-fuel booster on the top.

The workhorse of China's space programme until recently, Longmarch II, had two liquid stages, and has been used on nine occasions, with only one failure right at the beginning. Longmarch III, according to Zhang, has three stages, of which the uppermost is fuelled by hydrogen and oxygen; he says that it is comparable with the first version of Ariane 1 in that it should be capable of putting 1 tonne of mass into a low orbit in preparation for putting part of the mass into geosynchronous orbit. Improvements now being undertaken, Zhang says, will increase the masses by a half, so as to put 1 tonne, typically the weight of a communications satellite, where it can be useful.

The scientific programme has concentrated so far mainly on the measurement of energetic particles and cosmic rays. Chinese researchers with plans for satellites must first convince their institutes of the good sense of their proposals, whereupon the institute and its parent agency will seek to negotiate launching space.

Plainly, this process is as tortuous in China as elsewhere. Thus one of the boasts of the Guangzhou Institute of Electronics is that it has already finished work on the design and construction of the data acquisition kit intended for an astronomy satellite "soon" to be launched, and that it has already sent the equipment to Beijing.



A sextant, quadrant and celestial globe built by the Jesuits for the Qing emperors are on display at the Ancient Beijing Observatory, a massive gate that was once part of the city walls. Kublai Khan built the first observatory here in 1296. More recently, some extra "stars" were added to the celestial globe when it was pierced by Japanese bullets during the occupation of China.

Zhang, speaking for the Ministry of Astronautics, says that he knows of no plans to launch such a satellite.

Inevitably, the ministry employs technical people on a huge scale. Zhang estimates that its technical staff numbers 20,000, perhaps a quarter of them with university degrees. The ministry seems to have first claim on the output of graduates from a number of university departments as well as its own specialized college.

The ministry seems well aware that its chances of winning business in the market for launching satellites will turn crucially on its domestic success, whence the importance attached to the launching of a full-scale but still experimental telecommunications satellite early next year. The first television broadcasting satellite is now being built, but will not be launched until 1987 or 1988. There are also plans for meteorological and Earth resources satellites.

So is all this effort worthwhile, at least

at this early stage in the industrial development of China? One important spin-off, according to Zhang, has been that China has been required to set up factories for the manufacture of the special materials needed in the space programme, and in the process has acquired novel technology. The fly in this ointment is that many of the factories built for this purpose have been isolated from other general-purpose enterprises, but Zhang thinks that may change now that the government is doing everything it can to encourage mobility within the economic system.

Asked for a specific example of an innovation within the space programme that had found more general application, Zhang (after some thought) offered the case of the heat pipe (a way of transferring large amounts of heat by means of liquid metals), which is slightly reminiscent of the US NASA boast in the 1960s that the space programme had given the world the non-stick frying-pan. □



The aperture synthesis metre-wave radio telescope at Miyun, outside Beijing.

will soon have a station. A 25-m antenna facility for the 1–20 cm waveband which can link up to the world network is almost complete at Shanghai. As the only station in this part of the world it will be highly valued.

Solar studies, another relatively inexpensive area, and of practical use in predicting magnetic storms which disrupt telecommunications, are also well developed, at least in the more classical areas. New equipment coming into service includes a very fast recording solar heliometer telescope at Beijing University. A new telescope for observation of the fine-structure of the Sun is now complete at Yunnan Observatory and another will come into operation at Nanjing Observatory next year. Several major expeditions to observe eclipses have also been mounted and made good use of the unique observing conditions available in the Himalaya and Karakorum mountain ranges.

A unique site — in the Chaidamu Basin in the Gobi desert — has also been found for the new millimetre-wave telescope. It is quite high (3,000 m), flat (which makes it easy to do interferometry and to build roads) and very cold. That means there is very little water vapour in the atmosphere which makes for much higher resolution than comparable instruments at sea level. A 13.6-mm imported telescope will be run by the Purple Mountain Observatory and should be in operation next year. Here China can make major contributions for many molecular lines remain unexplored.

An attempt to fill an empty niche is also being made with the Miyun radio telescope. A set of antennas used for solar interferometry has been expanded into an aperture-synthesis array operating in the metre waveband (232 megahertz). Expensive and sophisticated centimetre-wave telescopes are already in place in the United States and United Kingdom so the telescope was designed for wavelengths they will not reach. Nor will the very large arrays now being built come to these wavelengths so Miyun should provide unique data for years to come. At present a two-year survey of the whole northern sky is under way. Any new variable stars, stars of unusual spectral type and the like that turn up among the 100,000 or so sources expected to be present will be studied in more detail later. □

Astronomy

Things could be looking up

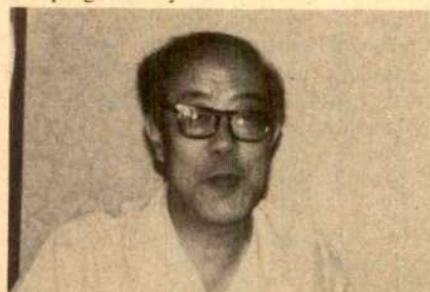
NEW astronomical facilities are appearing one after another in China: completed this or last year are a metre-wave and two solar telescopes; and nearly complete are a very long baseline interferometry (VLBI) station, a millimetre-wave telescope, a 1.2-m infrared telescope, and a 2-m optical telescope. But it would be an oversimplification to say that Chinese astronomy is booming.

As Professor Wang Shouguan, director of Beijing Observatory explained, the apparent burst of activity is more a matter of the Cultural Revolution having synchronized many projects. It was back in 1957 that a large optical telescope was first planned, for example. But then the Cultural Revolution came along and different parts of the telescope were slowly built at different places. Engineers are now just completing the daunting task of remodelling the parts to fit them together (which is "worse than doing it from new") and then the telescope — a 2.16-m reflector — will be installed near Beijing next year.

Major observatories have been built at Beijing, Purple Mountain (Nanjing), Shanghai, Yunnan, and Shaanxi (a relatively new time-service observatory) with the largest optical facility a 1-m telescope at Yunnan. But a new 1.2-m infrared telescope will be in operation at Beijing Observatory by the end of the year. Undergraduate courses in astronomy are given at Nanjing, Beijing Normal and Beijing universities and the Academy's University of Science and Technology at Hefei has an astrophysics centre for graduates.

Overall, China's astronomical facilities are quite modest. But that is not necessarily a bad thing. Professor Wang sees the immediate aim as training a corps of young

scientists, and for that small to medium-sized facilities are excellent because you have to "think hard to get the most out of them". When Chinese astronomers are more sophisticated they can try some really big projects. And even if more money were available Professor Wang's own view is that it should go on laboratories, computers and young people rather than big telescopes. That being said, China's facilities are really quite advanced for a developing country.



Professor Wang Shouguan, director of the Beijing Observatory.

High-level research has been carried out in time-service and positional astronomy where observing equipment is not so expensive and results of practical use can be obtained. Classical optical methods have been improved and China was first to develop an automatic photoelectric version of the Danjon impersonal astrolabe which truly eliminated observer error. Also on the drawing board (and attracting worldwide attention) is a 1.5-m astrometric reflecting telescope for the Shanghai Observatory. This exceptionally large telescope for astrometric work would enable the proper motion of faint stars to be studied photographically.

Much of such classical work is now being supplanted by VLBI and China too

Earthquake prediction

The calm before the storm?

A FEW weeks ago, with reports of the earthquake that killed more than 5,000 people in Mexico City still fresh, Professor Ding Guoyu of the State Seismology Bureau at Beijing confessed himself a worried man. The bureau and its Centre for Analysis and Prediction seems persuaded that there are alternating periods of seismicity (10–12 years long) and of quiescence in the occurrence of major earthquakes in China, and that a new period of activity is about to begin.

Notoriously, the period of the Cultural Revolution was marred by natural disasters, among which earthquakes were prominent, culminating in the loss of 240,000 lives in the earthquake near Tangshan in July 1976. Since then, Professor Ma Zongjim says, there was a period during which earthquakes of magnitude greater than 6.5 on the Richter scale were absent from China until this August's earthquake in Xinjiang province. So now there is a hurry to complete the network of seismic stations being thrown across China under the aegis of the bureau.

The generosity of the government's support for the bureau (with a budget of 100 million yuan a year) is conspicuous. The seismicity of China has two roots, the subduction of oceanic plates beneath the Pacific rim and the stresses and subse-

quent strains of the Earth's crust which are consequences of the collision between India and Asia.

Ding points out that China differs from seismic regions such as Japan in that a large proportion of its earthquakes are within the continental crust (and not buried in some oceanic trench as off the coast of Mexico). That continental earthquakes tend to be exceptionally damaging is easily understood, and is borne out by the evidence that four major earthquakes since 1932 have killed more than 750,000 people.

Accordingly, the plan is to cover China with a network of seismic stations whose function will be systematically to record smaller earthquakes in the hope that these will somehow be pointers (or forerunners of) catastrophe. There are four separate research institutes within the bureau, with its headquarters in Beijing, and a total of 24 provincial seismic bureaux intended to provide operational advice for provincial governments.

Although there are 400 seismic stations of various kinds in China, only 20 are at present equipped with standardized instruments, plus extensometers and tiltmeters. (The stations are also charged with collecting geomagnetic data, raw material for study of the secular variation of the

geomagnetic field.)

Altogether, the bureau has a staff of 5,000 technical people, recruited from university departments of geology and geophysics as well as from its own training school. Its managers say that the network they are developing cannot afford to omit any substantial part of China.

Ma, for example, points out that the earthquake of 1920 which killed 180,000 people had a Richter magnitude of 8.5 and yet occurred in a region which has since been apparently quiescent. It would be dangerous, the argument goes, to neglect such a region simply on the grounds that nothing much has happened in 65 years.

For the time being, the bureau is more an expression of anxiety about a recurring serious problem than a testimony to special Chinese expertise in earthquake prediction. The bureau, originally one of the Chinese Academy's institutes, was made independent on the recommendation of the State Council in 1980. It has to manufacture its own instruments (at a factory near Beijing) and is conscious that these are neither as sensitive nor as accurate as the US instruments being operated at Wuhan under the terms of a collaboration agreement with the United States.

The feasibility of earthquake prediction remains untested. The bureau says that when the immediate task of completing the seismic network is finished, it will try out all possible methods, including the use of accurate triangulation to monitor the movement of active faults. But during the recent quiescent period, the bureau has been looking for trends that may be meaningful in the historical records, which cover the past 2,000 years in reasonable detail.

As the world knows, there is one spectacular success on record for those who would predict earthquakes — the successful prediction of the earthquake near the city of Shenyang in the northern province of Liaoning, abutting the Korean border. This appears to have been an exceptional event, with a whole string of moderately sized earthquakes during the preceding two months converging on what eventually turned out to be the epicentre (see panel on left). Even so, the bureau can and does claim credit for avoiding the loss of more than 100,000 lives.

Just how, and when, the bureau will fulfil its mission of predicting a substantial proportion of the major earthquakes of China remains to be seen. But it works with the Institute of Soil Mechanics (of the Chinese Academy) on the design of buildings that may more adequately withstand major earthquakes. As in other fields, people are eager to learn whatever can be gleaned from other people's experience, to which end no fewer than 20 of the bureau's researchers will be at the forthcoming symposium on prediction, to be part of the annual meeting of the American Geophysical Union next month (December 1985) in San Francisco. □

Portents read in nick of time

THE circumstances of the Shenyang earthquake of January, which eventually was of magnitude 7.0, are unusual to say the least. The Qiang Shan peninsula, which forms the eastern boundary of the Bohai Sea, lies immediately to the east of an active fault running inland towards Shenyang. It is intersected almost at right angles by another, cutting across the base of the peninsula and the broad belt of thick sedimentary deposits supporting, among other things, the valley of the River Hun.

According to Professors Ding and Ma, the most conspicuous of the objective signs of trouble ahead were the small earthquakes preceding the magnitude 7.0 event by as much as two months. There were no fewer than 500 of these premonitory earthquakes in the five days before the major event, all of them within a narrow area centred on the eventual epicentre.

Other objective forewarnings included the variation of water levels in wells and dramatic changes in the output of oil from the oil wells drilled in the neighbouring sedimentary basin, which is a substantial source of petroleum.

There were also reports of strange animal behaviour, including the discovery that overwintering snails that would normally have stayed in hibernation were found to have ventured out and to have

been frozen to death and of deer that broke their legs by jumping fences too high for them. The obvious difficulty is that these abnormal behaviours, if accurately recorded, are as likely to be a consequence of the foreshocks as a sign that animals have a hidden sense of earthquakes on the way.

The Bureau of Seismology does not itself pretend to have an explanation of these phenomena, but says it has asked the Institute of Biophysics (under contract) to look into the question. The institute has a serious interest in neurophysiology, and seems a little sheepish about the whole affair, saying it will have to understand the animal nervous system much more fully before it can give a helpful answer.

No amount of scepticism on these points can rob the seismological bureau of the credit for having predicted an earthquake. But the procedure is far from simple. The bureau says that it does not itself have authority to publish earthquake warnings, only a provincial governor can take that responsibility. In January 1975, the provincial government was persuaded of the significance of the forewarning shocks by 10 a.m. one morning, eighteen hours before the earthquake finally came. It was a narrow squeak not merely for 100,000 people but for the reputations of the provincial government and the bureau. □

Cultural Revolution

True horror stories

ALMOST all those now working as scientists in China are people whose careers were launched before the horror of the Cultural Revolution began. Since the "smashing of the Gang of Four" in 1977, there has only just been time for a trickle of people to work their way through an undergraduate course, and through a PhD course on top of that, so as to become established in a job. The problem of "the missing generation" is apparent, and its implications widely discussed. But what happened to those now at work during that dreadful time? Sheer curiosity impels the question; politeness implies that an answer is usually forthcoming. Here are some case histories.

● One plant scientist spent a year working as a peasant in the fields 200 km away from the laboratory, a further year designing simple experiments to demonstrate the relative benefits of different ways of growing crops and then the whole of two years working on laboratory research with equipment transported from the main laboratory with the help of the "coordinating committee" set up in Guangdong by the provincial government.

● A mathematician (again in Guangdong) who had worked in the field of operational research spent the time of the Cultural Revolution working on the "optimization of industrial production" while travelling around factories in the region.

● An electrical engineer turned high-energy physicist worked on a project for constructing electricity supply lines.

● One mycologist was encouraged to spend several years scouring China for species and strains of soil fungi that might turn out to be sources of previously unknown antibiotics.

● A biochemist was allowed to spend the period of the revolution screening 20,000 people for the presence of alpha-feto-protein in their blood plasma, on the supposition that this might be diagnostic of the liver cancer (linked with hepatitis B infection) which is common along the eastern seaboard of China.

● One of China's most distinguished scientists, Professor Cao Tianqin, president of the Shanghai branch of the Chinese Academy of Sciences who, as director of the Institute of Biochemistry at Shanghai, organized the synthesis of insulin twenty years ago, tells the following horror story, without noticeable anger: "One day, they came and arrested me, my wife and my son. On the second day, they imprisoned me in the basement of the laboratory's animal house: for the next three years, my companions were the rats and the guinea pigs. I didn't see my wife during the whole of that time, and they wouldn't tell me how she was. That was especially worrying because I knew she was due to have an operation for cancer of the breasts. Only

after I was released did I learn that she had survived, but that her recovery from the operation had been very risky because the nurses and the physicians were fighting with each other, and had no time to look after the patients properly."

The moral, for those who would survive cultural revolutions anywhere, seems to be that one should at all costs avoid being a person in authority, for then there is no alternative to being labelled an enemy of the people. Those whose research has obvious practical application may, on the other hand, be able to stay in business. Even those whose research is somewhat academic may be able to persuade the revolutionaries of the opposite; appearances, in China's case, seem to have been more important than substance.

After the event, people seem uniformly to be more amused than offended by their experiences. Some even say that they have never been as healthy, and that it is beneficial that intellectuals should have the experience of working shoulder to shoulder with working people. Those who lost their lives because of the Cultural Revolution, are not available for comment.

Yet people do acknowledge that the ex-

perience of this period may have served some lasting purpose. Many agriculturally oriented research institutes are now more acutely aware of the need for what are called, in the West, extension services. And the notion that researchers should work hand in glove with industry, the cornerstone of China's plan for the future, is in the same spirit.

So may not the Cultural Revolution have been a benefit, after all? Faced with that question, an official of the Chinese Academy of Sciences at Guangzhou blurted out that there is nothing wrong in persuading scientists to work alongside peasants, but that sending researchers to the countryside during the Cultural Revolution was offensive "because the object was to wash our brains".

So will the experiment be repeated, or at least might the present opening to the outside be ended? There are three stock answers. One is that it is too soon to tell. Another is that "objective reality", a Marxist term, not peoples' inclinations will determine what happens in China. Yet another, given with much more enthusiasm in the south than the north, is that the process of unwinding has gone so far in the past eight years that it is no longer possible to turn back.

To outsiders, this absorbing question must still be wide open.

Electronics

Working for industry

THE Institute of Electronic Technology at Guangzhou puts out an advertisement to attract industrial partners that concludes with the exhortation "visit round our institute and have a promising business with us". The message is seriously meant and appears to be have been remarkably successful. In September, the institute was boasting that it had been able to sell a software package for the computer manipulation of Chinese characters to a commercial company in Hong Kong for HK\$100,000. One of these days, the product may appear in a Chinese word processor being developed by the Tensing Microprocessor Company, registered in Hong Kong.

The institute is also proud of its origins as a comprehensive research institute established by the provincial government in 1970, when China was in the grip of the Cultural Revolution. Then, it seems, the Guangdong government set up an organization to monitor the effect of the revolution on the infrastructure of its own operations, which is another mark of the sense of independence that flourishes in the south.

The institute became one of those belonging to the Chinese Academy of Sciences only in 1978, with a general remit to develop the techniques of telecommunications and of the applications of computers in this and other fields. With the general

adoption of the policy that government institutes must collaborate closely with industry, the institute has set up a commercial company, the Ling Nan Scientific and Technical Development Company, specifically so as to promote the transfer of technology developed at the institute as well as to provide design and computing services for industrial enterprises in China and elsewhere.

Inevitably, an institute in the field of electronics with Hong Kong on its doorstep must be growing quickly. There are now 400 people at the laboratory, four times as many as at the beginning. Since the institute's incorporation within the Chinese Academy network, much of its work has been stimulated by projects mounted on a national scale by the Academy. Thus the institute is the designer and has become the constructor of the telemetry packages used in the now extensive Chinese programme of balloon-borne physics, the cosmic-ray programme run by the Beijing Institute of High-Energy Physics, for example. Here, as in many other fields, the Chinese have taken great care that they will be able to recover in repairable form any equipment whose construction has been difficult and expensive. This same interest explains why the Guangzhou institute was chosen to build the telemetry and ground station equipment for an astronomy satellite being built by the

Academy institutes, but for which the launching plans are not yet known.

The institute is well along the road the Academy wants as many as possible to follow, that leading to close partnership with industry. Vice-director Chen Yan-yi says that dependence on central funds has declined from 90 per cent of the total budget in 1978 to just about a half now.

For the future, the institute expects that

it will be able to continue growing. Among other things, it is working with the regional government on schemes for the fuller application of microprocessors, not merely within the provincial government's sphere of operations but in Guangdong industry. Hong Kong's influence reaches beyond the special economic zone to the Pearl River, it appears. But the electronic technology institute still has a homespun

air. In a ground-floor laboratory, Wu Caiqing, manager of what is called the Chemical Arts Department of the Ling Nan company, uses what is described as a secret formula to produce etching-like patterns with artistic pretensions on the surfaces of useful and decorative objects of various kinds. Which only goes to show that, in the south, all ways of turning an honest penny are embraced. □

Science popularization

A CAST of thousands

WITH more than a million members, the Chinese Association for Science and Technology (CAST) is beginning to make a mark on the scientific scene. CAST is the umbrella organization for all of China's specialist scientific and technological societies or, as the Chinese would say, it is "a mass multidisciplinary organization of workers in science and technology". The association has only recently re-built its strength after being out of operation throughout the Cultural Revolution.

Those who have attended a conference in China will probably already know CAST for it plays a major role in organizing international exchange. Indeed, the dollars earned from visitors are all ready to help Chinese scientists abroad (when the ministry of finance eases its foreign currency restrictions).

But organizing conferences is only part of CAST's work. Its influence is now felt both by the government and the people. CAST regularly sends its policy recommendations, drawn up by specialist committees from its member societies to Party and government, and at the ministry's request gives technical advice.

CAST also spreads its roots down into local science and technology associations set up to bring science to ordinary peasants and workers. During the Cultural Revolution, there was little opportunity of learning about scientific ideas and CAST itself was disbanded — not until 1980 was it able to hold a national meeting again. But now the press is full of articles about peasants who made fortunes by increasing productivity through application of a little science. Some have achieved virtual stardom: like the chicken raiser in Heilongjiang Province who made so much money that she has been able to build a fifteen-room mansion. CAST is now out in force to take learning into the countryside.

Some campaigns are conducted on an awesome scale. Last year, in Hubei province, 20,000 CAST members went out and gave 80,000 workshops for middle school graduates from farming families — the total audience was five million people.

Keeping up the pace are numerous small scientific associations. At the county level its chief members are likely to be the local doctors, middle-school teachers and technicians from the bureau of agricul-

ture. (Such local improvement societies find their parallel in Victorian England.) Down at the village level (a level of organization formerly occupied by the commune) members will be those who have acquired some special technical skill, say in agriculture or cultivation of fruit trees. Some of their free time is spent in seeing their expertise disseminated. Local halls will be hired for lectures and on a market day a stand may be set up where villagers can learn about improved agricultural techniques.

There are nearly 2,200 science and technology associations at the county level, representing 93 per cent of China's coun-

ties. Some are even able to afford a few full time administrative staff. Down at village and commune level there are 41,000 sub-branches.

CAST does not actually control these lower-level organizations; they are all independent. What CAST does is to prepare publicity material, posters and books, provide speakers from its member societies and help coordinate national campaigns.

On a national scale CAST reaches a massive audience through magazines and books produced by its Science Popularization Press. And for children, there are science summer camps, "love science" campaigns aimed at encouraging kids to read scientific books, fairs showing scientific work by children and a national competition for child inventors. □



Some of the magazines produced by CAST's Science Popularization Press have circulations around ten million. The magazine Knowledge is Power (centre) carries some articles close to science fiction — like "Can man live for ever" and "Using hypnosis to stimulate creativity" — but the majority are educational: how electronic devices work; nutrient cycles on Earth, elementary BASIC and the like. Shown behind are regional science and technology newspapers with a more practical bent. They carry articles on such topics as new breeds of fish, forestry policy, how to produce carbon black, and the cross breeding of livestock. Alongside come some features designed to encourage: one story is of a 22 year old high school drop out who develops a new tea variety called "Rainbow" and lands a 7,000 yuan contract.

Aggregation by very large numbers

Computer models of aggregating systems have thrown a useful light on physical processes in the past few years. But too much computer power may make them more intricate than instructive.

THE neater the model, the more easily it is elaborated, and made complicated. That simple truth, always apparent, has been more evident than ever since computer power became more easily and cheaply available. The model of simple harmonic motion represented by the idealized simple pendulum could, no doubt, be made too complicated for a Cray computer to handle by the wooden elaboration of enough complexity.

Much the same, it seems, is now happening to the simple model of diffusion-limited aggregation first put forward four years ago by T. Witten and L.M. Sander (*Phys. Rev. Lett.* **47**, 1400; 1981). The original idea was simple, that of constructing a model to account for processes such as crystal growth whose rate is determined by the diffusion of aggregating particles through solution towards the growing crystal.

The simple version of the model is that in which aggregation takes place on a two-dimensional lattice. Particles are allowed to "diffuse" from the periphery of a piece of the lattice towards a central point by supposing that they move randomly, one lattice step at a time. It is necessary to specify the set of rules that determine when a particle will stick to the growing aggregate; most simply, it can be required to stay where it is whenever it reaches a lattice-site that is immediately adjacent to one already occupied.

Several important demonstrations have been possible with this simple, almost crude, model. Not least, for example, it turns out that aggregates constructed in this way are fractal structures; the numbers of particles they contain increase monotonically with increasing radius, but by some power of the radius which is less than 2.0, the value expected for a structure filling all the dimensions available in a plane. The value of the exponent in the power law with radius is now the familiar fractional dimension. The fact that just such behaviour is observed when dendritic solid structures grow from, say, solution, or when ice crystals form from the vapour, justifies the wide use made of the model and its elaborations in the past few years.

One side benefit of this simple model is that it lends itself easily to computer calculations. Tell the machine the rules, and it will replicate them indefinitely. So it has come about that a small army of ingenious people has been trying variants of the Witten-Sanders model. More than two

dimensions? No problem, except for the cost of computer time. What is the rate at which smaller aggregates will stick together to form larger structures? Again there is no problem once the rules are specified. The fact that the Witten-Sanders model also serves to account for phenomena other than diffusion-limited aggregation, percolation for example, has made it even more popular.

Naturally, there has been a great deal of interest in attempts to make the model realistic. Obviously, in a model growing by random aggregation, there is a possibility that loops will form between one part of the structure and another, thus excluding a large part of the accessible space from occupation. Are these physically realistic conditions, or should they be excluded as rare configurations with extraordinary implications?

Then the simplest set of rules, requiring that particles should stick to an aggregate whenever they reach a neighbouring site, makes no allowance for the likelihood that different sites might be occupied with different affinity. That is why some investigators have set out to calculate the probability that various sites will be occupied by running the random walk backwards.

More recently, attempts have been made to construct still more realistic models of aggregation by changing the rules, allowing a particle either to stick at the first nearest-neighbour site it reaches or, alternatively, to move to a neighbouring occupiable site, perhaps because such a site may be energetically more favourable. (Two neighbours are better than one.) There are reasons to suppose that the results of such calculations may enjoy a degree of realism (see P. Meakin and R. Jullien, *J. Phys.* **46**, 1543, 1985).

The most recent development is an attempt to construct a kind of equilibrium model of aggregation. The idea (due to R. Botet and R. Jullien of the University of Paris-Sud (*Phys. Rev. Lett.* **55**, 1943; 1985) is to begin with an aggregate of some kind and arrange that particles may escape from peripheral sites with single nearest neighbours, wander about by random walking (again on a two-dimensional lattice) and then stick to an aggregation site when next they reach it. If the wandering particle should get too far away, say to the circumference of a distant circle, it will be captured and released again at a random point.

Technically, there is no doubt that the

aggregating particle is stable. The number of particles is essentially constant; the rules specify that there can never be more than one particle on its travels. So what do the calculations show about the way in which the shapes of aggregates change with the repeated release and recapture of particles at the periphery?

First, for a specified number of particles in the initial aggregate, there is a final state with recognizable characteristics determined only by the number. For example, starting with a cluster of 100 particles allowed to randomize themselves by these formal rules, Botet and Jullien show that the radius of gyration of the end result is roughly eight unit lattice spaces. Attenuated structures (say, a string of 100 particles in a straight line) will finish up as a spindly structure. So too will structures that begin as compact clusters, with no spaces between occupied sites.

The obvious disappointment is that the model, as specified, is not a model for aggregates in equilibrium, but instead a model for an aggregate whose size has been arbitrarily fixed. Put simply, it is a model that presupposes that disaggregation is not an option for the system, or that the energy of the attachment of particles to aggregate is infinite. In the circumstances, especially because there can be no attempt to distinguish between the probability with which different kinds of clusters can occur, which is the same as saying that the energy of all configurations is the same. In other words, the model is a long way from being a model of equilibrium aggregation.

That does not, of course, imply that the calculations are without interest. But it is not surprising that the fractal dimensions of aggregates held constant under these conditions should be less than those of the aggregates formed in the first place by the Witten-Sanders process (1.54 against 1.70). Nor is it remarkable that some configurations built from very small aggregates (of, say, eight particles) should occur less frequently than sheer chance dictates. The promised calculation of what happens when larger aggregates split in two, by the severance of a bond chosen at random, may however be more directly relevant to speculation about the way in which aggregates rearrange themselves. The pity is that, for the time being, there are no usable analytical methods of making progress with these problems.

John Maddox

Epstein–Barr virus

Dream or reality of a vaccine?

from A.J. Beale

EPSTEIN–BARR virus (EBV) was first recognized in cultures of Burkitt's lymphoma cells and is a member of the herpes virus family. It was soon established as a cause of infective mononucleosis. There has also been a strong association between the virus, Burkitt's lymphoma and nasopharyngeal carcinoma. Since 1976, Epstein and his colleagues have mounted a systematic search for a vaccine against the virus in the hope that the viral aetiology of the two malignancies could be proved, with much benefit to high-risk populations in Africa, China and South-East Asia. Their studies have reached another landmark, as described on page 287 of this issue of *Nature*.

Epstein *et al.* have previously identified as a candidate immunogen an EBV glycoprotein termed gp340, and have devised means for its extraction, techniques for measurement of both gp340 and antibodies to it, and have developed an animal model of EBV-induced lymphomas in cottontop tamarins (*Saguinus oedipus oedipus*). The stage was set for the critical experiment in which vaccinated and unvaccinated tamarins were challenged with EBV. This experiment and the success of the immunization is now reported.

As the test vaccine, gp340 preparations, containing a minimal amount of residual living virus, were given to two tamarins in eight intraperitoneal doses at fortnightly intervals. Both animals developed antibodies that could be detected by an enzyme-linked immunosorbent assay, by immunofluorescence and by neutralization of EBV transformation of cord blood cells. They were both protected against a massive challenge dose of virus which caused disease and multiple tumours in control animals. Isolated gp340 in liposomes was less immunogenic than the membrane preparation: in a group of four animals given this vaccine, only one produced high concentrations of antibodies and was protected against viral challenge. Thereafter, two animals were given 17 intraperitoneal doses of the liposome preparation at fortnightly intervals and were subsequently challenged. Both animals developed transient inguinal lymph node enlargement after challenge; in one case this was accompanied by transient mesenteric node enlargement.

From the successful development of a vaccine against Marek's disease, to which fowl are particularly prone, it is already clear that a vaccine against tumours caused by herpes viruses is feasible, and the work now being reported on EBV shows that the development of a similar vaccine for a human herpes virus is theoretically possible. Is it likely to be a practical

proposition to make and test such a vaccine? The candidate vaccines of Epstein *et al.* are not practical: clearly, the membrane preparation of gp340 would need rigorous testing to show it was free of infectious virus before its use in man could be contemplated, and either vaccine would need to be presented in such a way that a protective immune response is achieved with fewer doses of vaccine. Doubtless the schedule could be improved to ensure priming and the elicitation of a secondary response.

The most important advance referred to by Epstein and his colleagues is the cloning and sequencing of EBV and identification of the sequences encoding gp340. It should therefore be possible to produce the protein in large quantities. Since it is glycosylated — more than 50 per cent of the mass is carbohydrate — it may prove best to prepare it in mammalian cells, as is done for many other viral immunogens, rather than in bacteria or yeast. Alternatively it could be expressed in vaccinia virus or some other carrier. The groundwork for developing a vaccine against EBV seems to have been soundly laid and the technology to produce sufficient immunogen is at hand.

The major problems of organizing and financing the procurement and testing of such a vaccine remain to be solved. The problems of organizing trials against diseases caused by EBV are formidable, even when compared with other herpes

virus infections. Whereas infectious mononucleosis is an early manifestation of primary infection, Burkitt's lymphoma and nasopharyngeal carcinoma are late manifestations that often take many decades to emerge. No one knows at present whether the late manifestations can be prevented after infection, but current opinion is sceptical. The aim, therefore, must be to prevent infection.

Trials of a vaccine against infectious mononucleosis are a practical and worthwhile proposition, given the will, in Western countries. Proof that EBV infection and this disease can be prevented by a vaccine would warrant the use of a vaccine to prevent Burkitt's lymphoma and nasopharyngeal carcinoma. It is doubtful whether it is practical to carry out a placebo-controlled trial lasting decades, but observation of the effect of vaccine on disease together with more limited trials of the vaccine on viral infections may suffice to demonstrate that EBV is indeed the cause of Burkitt's lymphoma and nasopharyngeal carcinoma.

For a number of diseases, ranging from malaria and pertussis to those caused by hepatitis B and EB viruses, there are now prospects for control by immunization, based on a molecular understanding of the immunogen required to produce protection. To harness this promise, a more determined and imaginative approach to preventive medicine and public health is required. Provided government agencies can see the economic as well as the health benefits of developing such approaches, the benefits to mankind and human health could be immense. □

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γ-ray sources

Does Geminga exist yet?

from Roger W. Romani and Virginia Trimble

THE peculiar γ-ray source in the constellation Gemini, termed Geminga, is once again puzzling astronomers. Originally found by the SAS-2 satellite¹ and later studied by γ-ray detectors both in space² and on Earth³, this object presents strange features in every wavelength band. One of the strangest — that it cannot be detected at visible and radio wavelengths — is reflected in its name, which also means "does not exist" or "is not there" in Milanese dialect. New optical observations (refs 4–6 and G.F. Bignami *et al.*, in preparation) have provided a tentative identification but these make Geminga seem even more inexplicable: as G.F. Bignami (Milan) reported at a recent meeting*, the object is exceedingly faint and

may have a very large proper motion.

As the brightest of the 20 unidentified γ-ray sources in the COS-B catalogue², Geminga is a natural subject of searches for corresponding sources in other energy ranges. The high count rate and large distance from the confusion at the galactic centre allowed the COS-B collaboration to obtain a position that is excellent by γ-ray standards. Images taken with the Einstein X-ray satellite's high resolution imager (HRI) and imaging proportional counter (IPC) led to the identification of Geminga with the bright source 1E0630+178 (ref. 7), supported, it then seemed, by the same 59–60-second, gradually lengthening pulse period in both X- and γ-ray data⁸. Buccheri *et al.*⁹ and others have doubted the periodicity, without necessarily disbelieving the identification, because there is unlikely to be an unusual

*NATO Advanced Study Institute on "High Energy Phenomena around Compact Stars" held at Cargèse, Corsica, 2–13 September, 1985.

X-ray source and a bright γ -ray source in one 0.4 square degrees of sky.

Searching at lower frequencies and with greater positional accuracies has met with some difficulty. Radio maps of the γ -ray error box (refs 10–12 and V. Boriakoff *et al.*, unpublished data) reveal more than a dozen 10–100 mJy sources, but there is nothing at the X-ray position and nothing particularly unusual anywhere.

At optical wavelengths, deep charge-coupled device exposures taken at the Canada–France–Hawaii telescope in January 1984 revealed a 21st magnitude image marginally within the Einstein error circle¹³. Dubbed the G candidate, it has been closely scrutinized by a number of groups. Discouragingly, optical and infrared photometry and spectroscopy (refs 4, 5, 14, 15 and M.J. Lebofsky, unpublished data) indicate that the candidate probably is just a G-type star — a cool white dwarf 100–200 pc from us or, more likely, a slightly hotter main-sequence or subdwarf star several thousand parsecs away. Both are inconsistent with the Einstein X-ray colour temperature of about 10^6 K (ref. 7) and the latter is also inconsistent with the very low X-ray absorption ($\leq 2 \times 10^{20}$ H cm⁻²), which indicates a distance less than 250 pc.

Moreover, a careful search for optical pulsations at a range of periods around those reported in X and γ -rays⁵, has set 2–4 per cent limits on any variability of the G candidate. The sky, according to Lebofsky, is full of similar stars and the chances of this one having anything to do with Geminga are slim.

Meanwhile, back at Lick Observatory, Djorgovski and Kulkarni⁴ have searched deeper at the HRI position and found two fainter candidates: G' at 24.5 mag near the centre of the error circle and G'' at 25½–26 mag off to one side. The lack of any brighter optical counterpart has interesting implications for 1E0630+178. The ratio of X-ray to optical luminosity is about 1,000 if G' is the counterpart, 2,500 if G'', and $\leq 3,000$ if neither is — among identified Einstein sources, only the radio pulsars and low-mass X-ray binaries have such large ratios. The absence of a detectable radio signal argues against a radio pulsar and the faintness of G' would place a typical X-ray binary at the improbable distance of 200,000 pc, arguing against an X-ray binary. This makes 1E0630+178 unlike any other known X-ray source, whatever association it has with Geminga.

G' and/or G'' seem to be peculiar, in any case. Djorgovski and Kulkarni⁴ report that G' has a tentative proper motion of 0.6 seconds per year; an alternative explanation is that this reflects a larger motion of G'' plus spillover of light between the images. In addition, the image that Bignami and his collaborators have obtained by summing the twelve exposures made in January 1984 shows a faint object that could be G''. But its position is very different from that found at Lick in

March 1985, implying a proper motion of 1.4 seconds per year for G'' and an upper limit of ≤ 0.2 seconds per year for G'.

Its rapid motion, if confirmed, must place the star quite close to us; for example, even a halo object moving at 200 km s⁻¹ would be no more than 30 pc away. What could it be? From the magnitudes, G' and G'' could be interpreted as brown dwarfs, not unlike the recently-discovered companion of ν B8 (ref. 16). But such a star is most unlikely to be either the X-ray source or a chance superposition; if there were a single brown dwarf so close to us in every 10⁵ Einstein error circles ($r = 3.3''$), it would make up the entire local 'missing mass' and can therefore be excluded from searches of larger areas.

It is somewhat more likely that G' or G'' is the thermal emission from a neutron star. Because the optical effective temperature could be anywhere from the X-ray colour temperature (10^6 K at the hot polar caps) to the X-ray effective temperature ($\sim 3 \times 10^5$ K), the implied distance is quite uncertain. Possible combinations are G'' with the lower temperature and a runaway velocity of about 200 km s⁻¹ at 20–30 pc, or G' with the higher temperature and atypical young star velocity of ≤ 50 km s⁻¹ at 80–100 pc.

Not even a white-dwarf binary companion^{17,18} could remain hidden at these distances, which leaves only binary¹⁹ or single²⁰ neutron stars as viable models. Either is possible in energetic terms, if the 60-second period is real and can be attributed to orbital motion or rotation in the two cases, although this then means γ rays must be grossly non-thermal. But both models imply soberingly short lifetimes, about 700 years, and the second may also

have difficulties in explaining the acceleration of particles to the energies needed to emit γ rays. The short lifetime would make sense if the 'guest star' of +437 gave birth to Geminga⁸ but the total absence of emission nebulosity in the vicinity argues against any recent local violence.

Clearly, one or two more deep images of the region, confirming or refuting the rapid motion of G'' and providing a bit of colour (temperature) information on the two candidates, will be critical for sorting out the confusion, in which Geminga, 1E0630+178 and G' or G'' could be one, two or three interesting objects.

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Plant sciences

Molecular view of pollen rejection

from Deborah Charlesworth

IN MANY species of flowering plants, a genetic self-incompatibility mechanism causes the rejection of pollen from the same plant. The genetic control is determined by one or more self-incompatibility (S) loci and it has been clear for several years that many of the mysteries surrounding the S locus will be resolved only when the molecular structure of the locus is known. A start in that direction is provided by J.B. Nasrallah and colleagues on page 263 of this issue¹.

Two types of self-incompatibility are known². In the Compositae and Cruciferae, and perhaps also in some other families^{3,4}, control of the pollen reaction is sporophytic — that is, the pollen type is determined by the genotype of the plant that has produced it — and there is usually, perhaps invariably, a single S locus. In gametophytic systems, which are known in several families and where the pollen

type is controlled by the alleles in the pollen grains, there is often one but may be two S loci, as in the grasses, or even more; *Beta vulgaris* has four⁵. With both types of system, there are very large numbers of alleles at the S loci, so that although the genetic data indicate that the S loci control the specificity of the reactions, which must be expressed both in pollen and in the stigma or style in order for self-incompatibility to result, there has been a recurring tendency to doubt whether the sequence information for all the S glycoproteins is really encoded at the S locus, and to suggest that some or all of it must exist elsewhere in the genome, and that the S locus somehow switches between specificities^{6,7}.

Nasrallah *et al.* already have some data relevant to this question. They have cloned DNA from the S₆ allele of *Brassica oleracea* and shown that the protein encoded by the S DNA is detected by an

antibody to the S_n glycoprotein produced in intact stigmas. Thus the S locus must contain the sequence that encodes the S_n glycoprotein, although it remains possible that other loci also contain S DNA sequences. A test of that possibility will be to determine whether all clones isolated from a given homozygous genotype contain the same DNA sequences, which would suggest that they are all derived from a single locus. Alternatively, the clones may reveal the presence of multiple self-incompatibility loci, even if they are very tightly linked. Nasrallah *et al.* have probed digests of the genomic DNA of homozygous lines with several S alleles with their S_n probe; although so far they have only used a single restriction enzyme, each allele has been shown to give a unique pattern, and the variation suggests multiple differences will be revealed by the use of further restriction enzymes. On the other hand, the results provide preliminary evidence suggesting that there is only a single locus; if there were many loci, one would expect to see a complex pattern of many bands, varying in intensity as well as mobility.

Another very important question that can now be studied is whether the S specificity is determined by the protein or the carbohydrate part of the molecule. The high number of S alleles in self-incompatible plant species makes it hard to imagine that the carbohydrate part alone could be responsible. Now that the DNA can be obtained, it will be easy to see how much the amino-acid sequences of the S proteins differ, and whether this variation is in the position and nature of attachment sites for the carbohydrates. Ultimately, it should be possible to look for regions concerned with the different functions of the S protein. At present, it is not even clear whether the three functions of specificity, and activity in the pollen and stigma or style are controlled by three separate, but tightly linked cistrons, as suggested by Lewis⁸ on the grounds that the three functions are independently alterable by mutation, or whether they could be under the control of a single locus with separate functional domains. Nor do we know whether the antigenic specificities used in detecting S proteins have anything to do with the regions that are important for the plants' recognition reactions.

A related puzzle is that apparently mutations of one S allele to another do not occur⁹. Except during another culture¹⁰, such changes have so far been detected only under conditions of inbreeding^{11,12}, thus if anything deepening the mystery. Since S loci are highly polymorphic, in all species where any relevant data exist, mutation must occur to balance chance loss of alleles. The rarity of S mutations might be because existing S alleles differ at multiple amino-acid sites. Assuming that only a subset of possible sequences of the S protein can function as self-incompatibility alleles, one would not be able to get

mutation from one S allele to another, but could get mutant types that lose the ability to be processed in pollen or style (as in Lewis's pollen-part and stylar-part mutations). Molecular studies should help to test these ideas, and should suggest why mutations are so rare.

Once the S gene is characterized in one species, it will be possible to probe for the S genes of related species, which will be of great practical significance. Indeed, it may be the best way of genotyping plants for the S locus, given the difficulty of genetic methods of testing and the uncertain success of techniques using the proteins, such as immunological methods^{13,17}.

Eventually, this type of work should enable us to determine whether S loci in different families of plants are homologous. The S DNA isolated by Nasrallah *et al.* will be useful with other sporophytic systems, but one can hope that DNA from species with gametophytic systems will be isolated in the future. It may then be possible to find out whether S systems are homologous in all angiosperms¹⁸, or have

evolved more than once, as seems likely from the distribution of the different types of system¹⁹. □

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Petroleum geochemistry

Advance in kerogen analysis

from R.P. Philp

It is commonly accepted that kerogen is a key intermediate in the formation of crude oil and/or natural gas from the organic debris of algae, bacteria, higher plants and other sources. Kerogen (Greek = oil or wax forming) is best described as the insoluble organic residue that remains after the organic solvent-soluble material, carbonates and silicates have been removed from the source rock or shale. Kerogen itself is formed following a series of complex chemical and, probably, microbial reactions involving the organic debris of higher plants, animals, algae, bacteria, spores, fungi, phytoplankton and zooplankton that is deposited in aquatic and reducing environments which favour the maximum preservation of organic material. Information on the nature of the organic source material responsible for its formation, its structure, the type of fossil-fuel products it will yield and its relative level of maturity is extremely important for petroleum exploration purposes. The paper by S.R. Larter and J.T. Senftle on page 277 of this issue reports a significant advance in the use of microscale pyrolysis techniques combined with gas chromatography (py-GC) for obtaining such data.

Most of the data used in oil exploration are based on analysis of oils and the soluble fractions of rocks. The kerogen in rock can be thought of as being indigenous, whereas it is always possible that the soluble fraction has migrated through the rock. The bulk of kerogen in source rocks and shales is insoluble in virtually all organic solvents and thus more difficult or

impossible to analyse by conventional methods such as gas chromatography (GC) or gas chromatography-mass spectrometry (GC-MS).

Microscale pyrolysis combined with GC and/or GC-MS provides a very powerful technique for kerogen characterization because a great deal of the geochemical data locked into the insoluble structure of kerogen can be released. Subsequent analysis of the pyrolysis products by GC provides information on the source material, thermal history and type of liquid products their host rock can generate. Another major advantage over analysis of the soluble material from the same rock is that all contaminants are normally removed during isolation of the kerogen, before characterization by py-GC. A major significance of Larter and Senftle's work is that it is a first attempt to quantify py-GC data for application to geochemistry. Equally important is the recognition of eight separate modes of kerogen composition from a number of cross plots involving normal hydrocarbon, aromatic hydrocarbon and total hydrocarbon yields obtained from the characterization of the kerogens by py-GC.

The word pyrolysis has been used in the geochemical literature to describe many different types of experiments, which has led to some confusion, to say the least. This is not the place to attempt a detailed classification but one broad distinction that needs to be made is between hydrous and anhydrous pyrolysis experiments. The work of M.D. Lewan *et al.* (*Science*

203, 897; 1979) greatly stimulated the use of hydrous pyrolysis techniques for evaluating the oil-generating potential of the source rocks from a particular basin. In this approach the source rock is sealed in a high pressure bomb with an appropriate amount of water and heated for varying periods at different temperatures. Obviously, water is not added to anhydrous experiments, but because crushed source rocks are very rarely completely free of water, it is doubtful if such experiments are ever truly anhydrous unless special conditions are observed.

Because of the apparent lack of alkenes in the products, the hydrous technique was promoted as the only method that could truly simulate the generation of crude oils in the laboratory, but it is more correctly thought of as a thermal simulation experiment in a closed system. In this situation, alkenes are unstable and are either transformed to saturated forms or react with the organic matrix. Evidence is, however, now available to show that alkenes can be generated under certain conditions of hydrous pyrolysis and their generation can be eliminated under certain conditions of anhydrous pyrolysis.

The most useful applications of hydrous pyrolysis are to simulate the effects of natural maturation in the laboratory and to determine the petroleum potential of a particular stratigraphic sequence at different burial depths within a basin. With one exception, the main application of anhydrous pyrolysis in geochemistry has been for typing kerogens and their possible precursors to determine the nature of the products that would result from the additional maturation or burial of the kerogen. The exception is the widely used Rock Eval pyrolysis method, widely used in the petroleum industry for routine and rapid screening of samples as they are recovered during the drilling of a well. In this method a crushed rock sample is subjected to a temperature range from ambient to 550° and the products analysed using a flame-ionization detector. Two chromatographic peaks are observed, the first relating to material normally extracted by solvents, the second to the products of thermal degradation of the kerogen. The temperature at which the second peak maximizes can be related to the maturity of the sample, and ratios of the two peaks provide information on the petroleum potential of a source rock.

Larter and Senftle report an advance in the technique of anhydrous pyrolysis, which commonly involves the combination of the pyrolysis system with either GC or GC-MS and permits fingerprinting of kerogens on the basis of the distribution of their pyrolysis products. (Although alkenes are produced during the pyrolysis reaction, this should not be looked on as a detrimental feature of the technique because alkenes still represent structural features of the kerogens which, in the natural situation, would be hydrogenated to the

corresponding alkane.) Larter and Senftle now describe how the use of polymethylstyrene as an internal standard permits the accurate quantitation of pyrolysis yields. The advantage of this approach is illustrated where two quite different macerals produce pyrograms that are indistinguishable from a qualitative point of view but can be differentiated by quantifying the yields of pyrolysis products. The study also shows that cross plots of the $C_7 - C_{25}$ normal hydrocarbons versus the $C_6 - C_8$ aromatics permit the different types of kerogens to be clearly observed. A further level of differentiation is obtained by plotting $C_9 - C_{30}$ normal hydrocarbon concentration versus the hydrogen index from the Rock Eval data (equivalent to H/C ratio), which in turn permits the differentiation of eight types of kerogen.

The past five years have seen tremend-

ous developments in the application of geochemistry to petroleum exploration in areas such as oil/oil and oil/source correlations, maturity, migration, source determination and basin modelling studies. Much of this has been based on solvent-extractable organic compounds. The next five years will see a similar expansion using of information from py-GC characterization of kerogens, together with partially insoluble material of high molecular weight, the asphaltenes. Microscale pyrolysis will be a key to eliminating confusion arising from the use of the soluble fraction; the quantitation described by Larter and Senftle will be valuable for the technique's continuing success. □

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Molecular evolution

The uniqueness of Archaeobacteria

from Roger A. Garrett

UNTIL recently, orders of unusual microorganisms that had adapted to a peculiar niche, or exhibited a biochemical oddity, were considered to be subdivisions of the prokaryotes. Superficially, this classification was tenable. But once genetic information, or nucleotide sequences, of the microorganisms were examined closely, some species were revealed to be as different from the other prokaryotes, or eubacteria, as they are from the eukaryotes. Consequently these organisms have been classified by Carl Woese and colleagues (*Science* 209, 457; 1980) as a third primary kingdom, the Archaeobacteria. Despite the evidence of their nucleotide sequences and several distinctive biochemical characteristics, this assignment has received a sceptical response from some quarters, but the participants of a workshop on the molecular biology of the archaeobacteria* should have been convinced by the plethora of unusual and unexpected characteristics reported.

The unique features of the nucleotide sequences have been demonstrated by Woese and colleagues for the 16S-like RNA molecule from the smaller of the two ribosomal subunits. They chose this molecule for at least two reasons: it occurs in every living organism and its functional role in protein biosynthesis has remained constant throughout evolution. Moreover, in the cytoplasmic ribosomes of eukaryotes, although its size increases substantially it retains the core structure common to the prokaryotic RNAs. Comparison of partial and later of whole RNA sequences produced coefficients for the degree of relatedness between organisms. It was these coefficients that first revealed

the deep divide amongst the prokaryotes.

This division quickly received support from biochemical studies. The membrane lipids of the archaeobacteria were shown to have ether-linked and branched aliphatic chains, whereas those of eubacteria contain ester-linked and straight aliphatic chains. Moreover, the DNA-dependent RNA polymerases from archaeobacteria yielded more complex subunit patterns than those of eubacteria.

The archaeobacteria consist of three main orders: the extreme halophiles, the methanogens that metabolize carbon dioxide to produce methane, and the sulphur-dependent extreme thermophiles that include the Thermoproteales (comprising the families Thermoproteaceae, Desulfurococcaceae, Thermofilaceae and Thermococcaceae) and the Sulfolobales that grow under very acidic conditions; only the species *Thermoplasma* does not fall into any of these orders but occupies an intermediate position. Each order has some special characteristics that reflect adaptation to a particular niche, as exemplified by the heat-stable RNA structure in the extreme thermophiles, but other characteristics are common to all archaeobacteria.

Among the archaeobacterial characteristics reported at the workshop were uniquely modified nucleotides in tRNAs, unique features of the secondary structures of each of the ribosomal RNAs, different ribosome shapes and sensitivities to antibiotics, DNA-dependent RNA-polymerase subunits that seem to be like those of eukaryotes, unique reverse gyrase that produce positive super-coils in closed circular DNA and nucleosome-like structures; there was also evidence of unusual modes of gene transfer.

*EMBO Workshop "Molecular Genetics of Archaeobacteria", Martinsried, West Germany, 23-25 June, 1985.

The instability of the genome of *Halobacterium halobium* and of closely related halophiles received special attention. Recent studies of its chromosomal organization and of various extra-chromosomal elements (W. Goebel, University of Würzburg; and F. Pfeifer, University of California, San Francisco) shed some light on the structural basis of the instability. The main chromosomal fraction, FI DNA, is 68% G+C-rich and can be readily separated from the more A+T-rich FII DNA fraction. The latter is complex and contains a heterogeneous collection of covalently closed circular DNAs, including a large plasmid carrying many insertion elements, as well as various minor components. Also present in the FII DNA are non-circular 70 kilobase (kb) 'islands' that probably associate with the chromosomal FI DNA; these also contain inser-

tion elements and participate in the genetic variation. A phage, ϕ H, isolated from the halophiles, also exhibits a high degree of genetic variability. No conclusive explanation was offered for the functional significance of the lability of the *H. halobium* genome, although some genomic changes seem to be reversibly inducible by changes in external salt concentration.

Exciting developments were reported in the characterization of virus-host systems (W. Zillig, Max-Planck-Institut für Biochemie, Martinsried) that will prove valuable in future for investigating the molecular genetics of the archaeobacteria. They included the nucleotide sequence of the virus-like SSVI from *Sulfolobus solfataricus* that has a 16-kb genome, and the identification of four viruses from *Thermoproteus tenax*, that partially resemble eukaryotic viruses in their structural orga-

nization. The ϕ H phage and the plasmid pSL10 from *S. ambivalens* are especially suited to vector development; other potentially important phage and plasmid vector systems were described for the methanogens.

The regulation of gene expression also figured prominently, with emphasis on the more readily isolated rRNA and tRNA genes and the bacterio-opsin gene of *H. halobium*. The observation of multiple transcriptional starts for the single rRNA operon of *H. cutirubrum*, occurring at regularly repeated sequences that include the motif 5'-A-A-G-T-A-A-3', was unexpected (P. Dennis, University of British Columbia, Vancouver). Another putative promoter sequence 5'-G-A-A-N-T-T-T-C-A-3' was deduced from sequencing the *purE*, *proC*, *hisA* and *argG* genes of various methanogens expressed in *Escherichia coli* (J. Reeve, Ohio State University, Columbus). Termination sites are less easily found; although some potential signals resemble those of eubacteria, others do not — it was suggested that five consecutive thymidines might be sufficient for ending the methyl-CoM-reductase gene of *Methanococcus voltae* (A. Klein, Phillips University, Marburg).

The detection of introns among the extreme halophiles and in a thermoproteale was a surprise. A 105-bp intron has been found in the tRNA^{Trp} gene of *H. volcanii* and other halophiles, with a fairly well-conserved sequence (C. Daniels and W. Doolittle, Dalhousie University, Halifax). Moreover, a 622-bp intron containing an open reading frame has been located in the 23S rRNA gene of *Desulfurococcus mobilis* (J. Kjems and R. Garrett *Nature*, in the press) in a similar position to that of an intron in the corresponding gene of the lower eukaryote *Physarum polycephalum*. Although no introns have been reported for protein messenger RNAs, the new results, together with an earlier report of putative small introns in the tRNAs of the sulfolobales, suggest that the presence of introns within ribosomal and tRNAs constitutes another fundamental difference between archaeobacteria and eubacteria.

Various disputes arose concerning archaeobacterial origins and centred on the order in which the extreme halophiles, methanogens and the extreme thermophiles separated from one another and from eubacteria and eukaryotes. The proposal by J. Lake (University of California, Los Angeles) that the sulphur-dependent thermoproteales and sulfolobales should be considered a separate kingdom because a few of their biochemical properties differ markedly from those of the extreme halophiles and methanogens received an extremely negative reaction, as did his contention (Lake, J. *et al. Proc. natn. Acad. Sci. U.S.A.* **82**, 3716; 1985) that the extreme halophiles should be grouped with eubacteria because their ribosome shapes are compara-

The loess region of China

DELEGATES from ten overseas countries recently attended the international symposium on loess research held in Xi'an, in the People's Republic of China (5–13 October, 1985), the first such meeting to be held in the classic Chinese loess region.

A 5-day field excursion before the meeting traversed several loess regions, distinguished by their geomorphological features, between Xi'an and Ansai, 400 km to the north. Our route crossed the broad Wei River flood plain which is mantled by loess and dissected by a few small gullies. Northwards, the hill country is dominated by long, loess-covered ridges termed liang. Limestone quarries along the road gave a unique opportunity to observe how the liangs are related to the underlying bedrock. Smaller, loess-covered hills, known as mao, separate this area from the flat-topped loess plateau, termed yuan. Here the loess is about 100–200 m thick and is extensively cultivated with wheat and maize, a little cotton and tobacco.

The most spectacular features of these yuans are the deep erosional gullies that dissect the plateau. In particular, near Luochuan, the gully is some 130-m deep and in the near-vertical cliffs a sequence of more than 15 palaeosols can be seen overlying the basal red clay. One site has been studied for about 30 years and the chronological sequence has been established primarily by palaeomagnetic measurements of a core nearby. These results imply that the 130-m deep section spans the last two magnetic epochs, the Brunhes and the Matuyama. Detailed magnetic stratigraphies reaching back into the Gauss normal epoch (Heller, F. & Liu, T-s *Nature* **300**, 431; 1982), indicate that loess deposition may have started as early as 2.66 Myr ago. This is similar to the 2.9 Myr date reported by G.J. Kukla (Lament-Doherty, Geological Observatory) for the beginning of

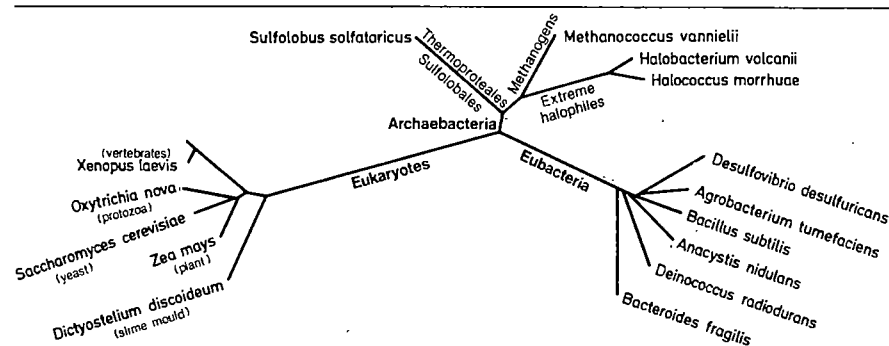
non-biogenic silica incorporation in a marine core off the Japanese coast. Similar and more continuous records are expected from the Qaidam Basin, closer to the source area of the loess, and it is tempting to correlate the palaeosols with the interglacial periods revealed by the deep-sea oxygen-isotope record.

Another feature of the symposium was the integration of geotechnical and chronological studies of loess. A large number of physical properties, such as the bulk dry-density and liquid limit, have been measured and related to the probability of landslides and collapse of loess when it is waterlogged. These properties are of interest as the area is bounded by major active faults and records document many major earthquakes in the past two thousand years.

A highlight of the field trip was a visit to an experimental conservation area, in Ansai County, Shaanxi Province, set up in 1980 by the Northwest Institute of Soil and Water Conservation of Academia Sinica. Erosion of the loess and subsequent landslides are major problems for agriculture, road maintenance and the safety of villages. Experiments designed to measure water run-off and removal of loess have been set up on the top of a hill overlooking the field station, and different types of shrubs and trees have been planted to try to stabilize the surface. We are also privileged to be the first foreigners to visit a village where the results of this research have been applied. Higher crop yields and more terracing have enabled the villagers to cover the upper slopes with trees and leguminous plants, which protect their livelihood.

Ann Wintle and Edward Derbyshire

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Universal phylogenetic tree compiled from completed sequences of 16S-like RNAs. The lengths of the lines indicate sequence distances and are an approximate measure of evolutionary time. The figure was kindly provided by G. Olsen (Indiana University, Bloomington) and C. Woese (University of Illinois, Urbana).

ble and because it is unlikely that photosynthesis evolved in two separate events. In response, Woese (University of Illinois, Urbana) emphasized the importance of considering many biochemical characteristics when constructing phylogenetic trees and stressed that these problems should continue to be resolved at a molecular level. He then presented, for the first time, a universal phylogenetic tree (see the figure) based on, recently completed 16S-like RNA sequences. The lines indicate the relative sequence distances between the archaeobacteria, eubacteria and eukaryotes but the root of the tree is not defined. Although he is convinced that 16S RNA is an excellent chronometer for evolution because of its universality and slow rate of change, Woese recommends that RNA sequence data should be supplemented by pro-

tein sequences including those of the subunits of the DNA-dependent RNA polymerases and of a protein not directly associated with the genetic apparatus, such as an ATPase.

There were optimistic messages for all participants. The biotechnologists were promised heat-stable enzymes from the extreme thermophiles and genetically engineered methanogens for improving industrial processes. The molecular geneticists were offered a wealth of novel and exciting problems. And the evolutionists were shown how to refute the age-old prejudice that first there were prokaryotes and then eukaryotes, and how to infer the nature of early forms of life on Earth. □

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Behavioural ecology

The limits to infanticide

from Paul H. Harvey and Anna Marie Lyles

THE true extent of infanticide among mammals is, it seems, only slowly becoming apparent. Over the years, new evolutionary explanations have been given to account for the limits to infanticide as currently perceived, but then a species crops up that breaks the rules. A new record of grisly proportions has been set by John Hoogland's observations on a wild population of black-tailed prairie dogs (*Cynomys ludovicianus*) in South Dakota¹. Offspring in more than half of the prairie-dog litters he observed are killed, usually by close kin.

Classical ethologists, such as Lorenz², argued that animals rarely kill others of their own species. Laboratory studies eventually showed that this was not true but, it was then claimed, laboratories are unnatural environments and infanticide is merely a maladaptive or pathological response to high population densities³. More recent field evidence has revealed that infanticide may be a predictable event in some natural populations. For example,

male lions (*Panthera leo*)⁴ and langurs (*Presbytis entellus*)⁵ entering a group regularly kill the young that were fathered by the males they replace.

Hrdy⁶ outlined a number of reasons why animals might kill young of their own species: the infants could represent a scarce resource, such as food (exploitation); the death of infants might increase the availability of other resources to its killer or relatives of its killer (competition); eliminating the dependent offspring of a possible future mate might increase the opportunities of mating access (sexual selection); parents might even be selected to kill particular offspring if investment in them was not likely to be worthwhile (parental manipulation); finally, some infanticide might not have adaptive significance and the eventual reproductive success of the killer could ultimately be reduced (social pathology). Competition and sexual selection account for most infanticide recorded among mammals⁷.

The main message of the most recent

News and Views article on infanticide⁸ was that, other things being equal, individuals are more likely to be selected to kill unrelated than related young, because the latter may be partially protected by kin selection. Indeed, it has been considered that, among mammals, close relatives rarely commit infanticide. In a book published last year, the editors wrote "For most mammals, however, with the exception of humans where mothers in certain circumstances may opt to abandon offspring, infants tend to be killed by unrelated individuals"⁷. Thus, although lion and langur males kill the young of their displaced rivals, thereby bringing the females back into oestrus more rapidly, the related females in a group do not kill each other's offspring, nor do males kill the young that they or their relatives are likely to have sired. Similarly, Sherman states that among Belding's ground squirrels (*Spermophilus beldingi*), "individuals never killed young of relatives (that is, cousins or closer kin)" even though "at least eight per cent of all young born were preyed on by conspecifics"⁹.

Hoogland's results¹ are a stark contrast. Out of the 73 black-tailed prairie dog litters that he observed, 51 per cent lost either some (13 per cent) or all (38 per cent) of the offspring. Most of these were killed by lactating females, 80 per cent of which were close relatives — mother, daughter, sister, aunt, niece or cousin of the victims' mothers.

Why might natural selection favour killing the offspring of a relative? Hoogland suggests that infanticidal females, which he terms 'marauders', acquire valuable food by cannibalizing the young they kill (exploitation as defined by Hrdy⁶), which helps them with the successful weaning of their own litters. In support of this idea, Hoogland shows that marauders are in better condition than non-marauders when their young are weaned, and they wean more and heavier offspring. But there are also costs: prairie dog females live in burrows alone with their young and when a lactating female is out marauding, her offspring are left undefended. Three



A prairie dog (photography courtesy of the Zoological Society of London).

females lost their own young when committing infanticide elsewhere and two more lost theirs while visiting burrows where infanticide had recently occurred. Given these costs, infanticide is most likely to pay dividends if it involves nearby burrows. The problem is that nearby burrows are usually occupied by close relatives. Prairie dogs group into coterie, consisting of female kin with an unrelated adult male. All members of the coterie defend their territory against invasion from outsiders. Attacks on distant burrows occupied by non-relatives therefore entail greater risk in both time (absence from own burrows) and danger, since a foreign coterie's defences have to be breached, as well as those of an individual burrow. Possibly, the extra benefits in genetic terms may not be worth the extra costs. □

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Charles F. Richter (1900–1985)

CHARLES Richter, co-developer of the earthquake magnitude scale which bears his name, died on 30 September, 1985. He lived in California most of his life and that is where he developed his fascination with earthquakes during the embryo stages of the new science of seismology. In 1927, at Robert A. Millikan's request, he accepted a position as a physicist at the newly established Seismological Laboratory which was later to become part of the California Institute of Technology. During the 1930s he collaborated with Beno Gutenberg on a series of seminal publications entitled *On Seismic Waves* published between 1934 and 1939 in *Beiträge zur Geophysik*. These studies laid the ground work for modern observational seismology and resulted in the first detailed models of the mantle and core.

This collaboration also led to various earthquake magnitude scales and to the first complete compilation of earthquakes, published as *The Seismicity of the Earth* in 1954. Richter's book *Elementary Seismology*, which appeared in 1958, is an encyclopedia of earthquake information and is still widely used. Don Anderson

Neuroanatomy

Antibodies to acetylcholine at last

from Felix Eckenstein

IN a recent publication, M. Geffard and colleagues describe the production of antibodies to the neurotransmitter acetylcholine (ACh) and the successful use of these antibodies for the immunohistochemical localization of ACh¹. This is a remarkable breakthrough in the effort to identify cholinergic structures in the nervous system; the more so as it has been held for a long time that ACh cannot be fixed in tissue, so that the molecule cannot be localized *in situ*. Geffard *et al.* have overcome this problem by using an ingenious way to modify ACh chemically within the tissue to a form that can be fixed and localized by antibodies prepared against ACh that has been modified in a similar way *in vitro*.

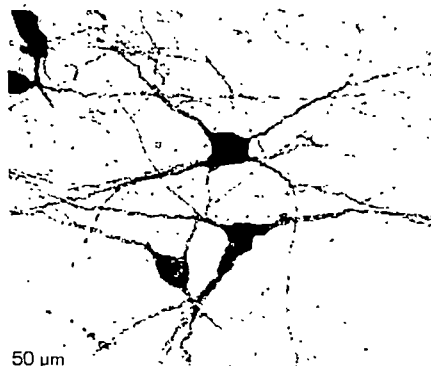
Acetylcholine was one of the first neurotransmitters to be described and the vertebrate neuromuscular junction, the classical example of a cholinergic synapse, is probably the system where the physiology of synaptic transmission has been studied in most detail. But our understanding of the systems of neurones in the central nervous system that use ACh as a transmitter is much more limited. The work of Geffard *et al.* adds an important new method to those already used for the anatomical identification of neurones that use ACh as their transmitter.

Up to now, the methods for the anatomical identification of cholinergic neurones have included the histochemical localization of acetylcholinesterase (AChE), the enzyme involved in the hydrolysis of ACh^{2,3}; immunohistochemical localization of the enzyme for synthesizing ACh, choline acetyltransferase (ChAT)^{4,5}; or dry autoradiography to localize sodium-dependent high-affinity choline uptake (HACU)⁷. It has become clear, however, that AChE is also present in a variety of non-cholinergic neurones^{8,10}, which limits its usefulness as a marker. Localization of HACU is technically diffi-

cult and is also not entirely specific because non-cholinergic cells, such as rabbit photoreceptors, display HACU¹¹. So far, ChAT has been the only reliable marker for cholinergic neurones, although controversy about the specificity of antibodies to ChAT has plagued the field for a long time¹². Now that pure ChAT and monoclonal antibodies against the enzyme have become available^{4,6}, ChAT-immunohistochemistry is very useful for identification of cholinergic neurones.

In addition to confirming results obtained with antibodies to ChAT, the immunohistochemical localization of ACh offers other advantages. First, due to the limited cross-reactivity of ChAT-antibodies from different species, cholinergic neurones could be identified by this method only in vertebrates, whereas antibodies to ACh should have broader cross-reactivity. Second, the method for the preparation of antibodies to ACh described by Geffard *et al.* is simple enough for others to follow easily, whereas the preparation of antibodies to ChAT requires cumbersome purification of the enzyme.

One potential problem of the method lies in the way ACh has to be modified to allow its fixation. This step involves the substitution of the acetyl group of ACh with another group that is subsequently fixed in the tissue. It seems possible that this method will not only identify cholinergic neurones but also structures containing other choline metabolites. The rabbit photoreceptor, because of its high choline metabolism, should be a good model for testing this reservation. On the other hand, it is encouraging that the staining pattern obtained by Geffard *et al.* closely resembles that seen with ChAT-immunochemistry (see figure). This novel method is a more than welcome addition to the field, and one that is likely to add considerably to our understanding of the organization of cholinergic systems. □



Photomicrograph of ChAT immunoreactivity in 30–40 μm diameter multipolar neurones in the basal forebrain of the rat. In this area, neurones of similar morphology have been labelled by Geffard *et al.* using their antibodies to ACh.

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Catastrophism is still viable

SIR—It is true, as Weissman states¹, that Innanen *et al.* proposed that the Sun's galactic motion might correlate with geophysical periods. Similar claims have been made over the years for ice ages, mass extinctions, the Earth's magnetic field reversal frequency, the extrusion of carbonates in Canada and so on. Indeed Holmes some fifty years ago, without the benefit of radiometric dating or the plate tectonic revolution, pointed out that there were 30 Myr and 250 Myr cycles in the geological record. These long-standing claims, largely neglected and only now being rediscovered, were an important element in our conception of the galactic theory of terrestrial catastrophism. But what was missing from all the papers before 1978 was a *mechanism*. And although it seems to elude Weissman, a new idea has emerged, namely that the well known periodicities in the terrestrial record may arise because of the dominating influence of giant comets on the Earth's evolution and of the molecular clouds in the Galaxy in turn on their intermittent influx (see ref. 2 for a review, including references therein). Giant comets in particular take precedence because they contain most of the incident mass: the prolonged effects of the dust input from giant comets may therefore compete with the prompt effects expected from bombardments by their larger debris.

There have been claims recently of course that the mechanism does not work on the grounds that the Sun, unlike other stars of its age, must remain so close to the galactic plane that it cannot yield any significant modulation of the comet flux^{3,4}. However, such claims neglect the fundamentally stochastic nature of the galactic interaction, affecting the Sun's orbit as well as the comet cloud. Thus the current solar orbit is anomalously flat, tending to depress the 30 Myr component, but it experiences large gravitational deflections including a probable memory-erasing disturbance during the very recent encounter with Gould's Belt⁵. Terrestrial cyclicities can only be avoided therefore if the Sun avoids molecular clouds, an eventuality that now appears to be hardly realistic.

By the same token it is misleading to favour stochasticity⁶ at the expense of cyclicity. It has been argued⁶ for example that the occurrence of large sea regressions immediately preceding the Permian and end-Cretaceous extinctions is too large a coincidence to swallow on the impact hypothesis. This comment is only applicable to the stray meteorite hypothesis⁷ however and would not apply to the galactic theory — we have ourselves pointed out that the two great mass extinctions were each preceded by the onset of a strong mixed magnetic interval. Thus the meteorite hypothesis predicts no cyclicity

ties and, like Nemesis, seems to owe its erstwhile popularity more to Californian hype⁸ than the real astronomical environment.

Whilst it seems therefore that Weissman's quixotic comment has little bearing on the status of the galactic theory of terrestrial catastrophism, the question of how the Earth responds to the huge astronomical perturbations that are now expected remains one of formidable complexity, hardly yet tackled in a quantitative fashion, and to that extent one might agree that catastrophism is as yet unexplained.

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Tenuous evidence for the luminous mouthed shark

SIR—The capture of a second specimen of the 'Megamouth' shark *Megachasma pelagios*¹ has prompted further discussion of its feeding mechanism by J. Diamond in a *News and Views* article². The published description of the first specimen³ noted that the lining of the mouth had a silvery appearance with pore-like structures and reported that "At the time [24 hours after its capture] it was speculated that these might be luminescent organs, but we have no evidence for this". These initial speculations were given more prominence in an earlier popular article⁴. The account of the second specimen describes the upper jaw and palate as "remarkably iridescent"⁵ and the previous speculations have been revived and developed by Diamond, who makes a number of assumptions in support of the premise that Megamouth has a luminous mouth into which the prey (euphausiid shrimps, copepods and jellyfish) are attracted. Beguiling though such a hypothesis may be, its factual basis is too tenuous for it to be allowed to go unchallenged.

The two specimens have been captured at epipelagic depths (< 38m and 165m) yet their "eclectic combination of habitus characters"³ suggest they are relatively weak swimmers. Diamond infers from this that they live "well below the rich plankton zone" and cannot support themselves by direct filter-feeding, unlike the whale shark and basking shark. He infers further

that because the prey is itself bioluminescent it would be attracted to an illuminated mouth. There are few grounds for either inference. The nighttime biomass peak of the micronekton in general and Megamouth's euphausiid diet in particular, lies in the upper few hundred metres, rather than right at the surface. Nor is there any direct evidence to suggest that euphausiids are attracted to a bioluminescent source. There is really no basis for speculation that the mouth is bioluminescent other than its unusual iridescence. Even so, although some luminous organs are silvery it does not follow that all silvery tissues are luminous. It could equally (perhaps more plausibly?) be argued that a reflective lining to the upper part of the mouth might make it less conspicuous than a wholly dark maw, and therefore more efficient in any daytime filter-feeding.

Like Diamond, I do not know of any filter-feeder that uses light to attract plankton (although it was once suggested as a function for the luminescence of the piddock, *Pholas*⁶). The answer to the question he poses of why filter-feeders do not attract prey into their mouths is probably a function of the difficulty of generating a non-specific signal of sufficient potency and range to supply the dietary needs. It is more likely that the problem of Megamouth's feeding methods will be solved by better understanding of both its sensory abilities and its jaw and filtration mechanics. The extraordinary nasal capsules, and the suggestion that Megamouth is more closely related to the whale shark than originally suspected⁶, may both be relevant in this respect.

The analogy drawn by Diamond, between the fishy example of Megamouth's proposed pelagic moth-trap and the feathered one of the frogmouth's nocturnal fly-paper, is certainly entertaining but probably more fabulous than factual.

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Down to the roots

William Coleman

The Background of Ecology: Concept and Theory.

By Robert P. McIntosh.

Cambridge University Press: 1985. Pp.383. £30, \$39.50.

ECOLOGY began in confusion and then prospered in confusion. At the outset an interest of very different enthusiasts, ecology has retained to a singular degree a seemingly irreducible diversity of subject matter. There has been much talk of theory, and the hope that one or another proposed theoretical programme would at long last provide the foundation for effective unification of the ecologist's concerns. That happy moment has not yet arrived. Each ecologist, it appears, is fated to seek his own, often highly personal view of the science, and not infrequently history has been used as a guide in that quest.

This situation is not unique. From classical antiquity to the nineteenth century, medicine, riven by conflicting theories of disease, turned to historical narrative and analysis in order to seek doctrinal coherence for purposes of further investigation and, above all, to afford the beginning student a feeling for the principal concepts of medicine and a sense of the structure of the profession. Chemistry followed a similar course. Prior to the revolution carried out by Guyton de Morveau, Lavoisier and Dalton, from which resulted a wide-reaching theoretical framework based on the regularities of combining proportions and a transformation of chemical language, the chemist often used a historical account of chemical investigations to give shape to his discipline and to impart to newcomers a sense of its accomplishments and problems. History was thus an integral part of medicine and chemistry, not an idle ancillary activity.

It appears that Robert P. McIntosh is following a similar course. *The Background of Ecology* is divided into eight chapters, arranged thematically. All revolve about the author's main concern, the rise of "self-conscious", that is, empirically and theoretically self-aware ecology, and each tends to offer in itself a complete chronological account. This procedure entails occasional but not objectionable repetition. There is, however, another and more serious drawback to McIntosh's approach. The structure of the book dictates an episodic view of the development of ecology. The topics covered — the emergence of early ecology from natural history, dynamic/community ecology, quantification and population ecology, ecosystems and systems ecology, the awesome expectations of theoretical ecology, and ecology as popularly understood (that is, conservation and the environmental movement) — appear as if on parade,

each in multi-coloured costume and announced by contentious advertising. While McIntosh is a spectator of this parade, indeed, a very attentive and extraordinarily well-informed observer, he only occasionally views the proceedings with a sharply critical eye and even more rarely seeks to relate to his readers the meaning of the great display.

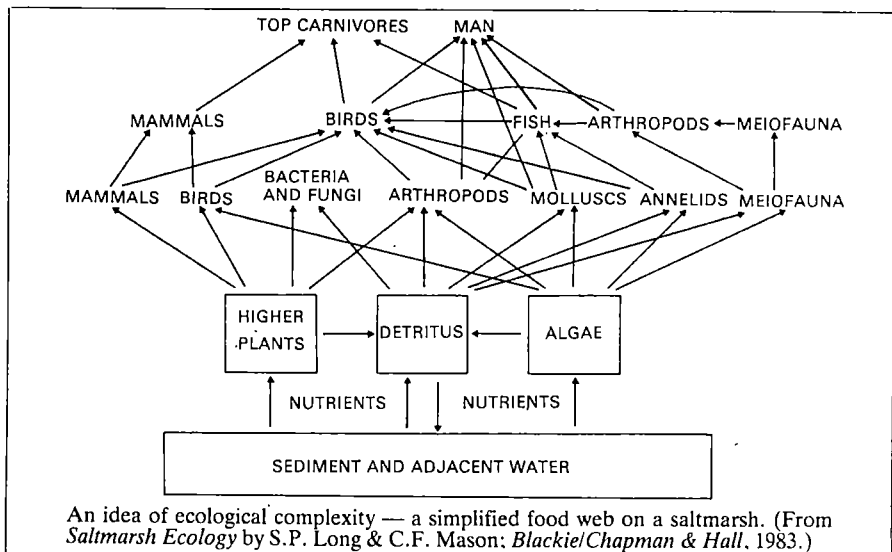
The Background of Ecology offers, in fact, a remarkable tally-sheet or taxonomy of who thought what and when, and who rose in opposition; in this lies one of its many virtues. It gives, however, little indication of why such opinions were entertained or rejected; what is largely missing from the book is a sustained analysis, an interpretation of ecology's history. Lacking this, we can conclude only that ecology has an old and respectable background and that, in more recent years, the science has pursued countless disparate objectives, all the while never being able to settle on a set of widely acceptable goals.

Of course, ecological ambitions have always been large and there is dubious merit in chiding an observer of the development of a science for not accomplishing what few of its practitioners themselves have accomplished. To early ecologists, as to their successors, it was relatively easy to state the needs of the science. Kerner von Marilaun, for example, announced in 1863 that the distribution of plants "follows immutable laws. Every plant has its place, its time, its function and its meaning". One's task followed directly therefrom: discover these laws. Nothing less would do for animals and, ultimately, for

the combined biotic world and for its relationship to the non-living world. Self-conscious ecology largely began with ambitions such as those expressed by Kerner. Botanists, exploring the spatial distribution and physiological adaptations of plants, especially the former, laid foundations for the science and created its first large-scale generalization, the notion of succession. Limnologists and marine scientists also stressed the idea of community, noting not only distinctive assemblages of organisms but emphasizing more than others the myriad interactions that bind these organisms together.

Until the 1920s, this was the primary fare of the ecologist. New possibilities then emerged. Under the protection of Raymond Pearl, and developed by Lotka, Volterra, Gause and others, the logistic curve and then two-species interactions swept animal ecology. The population now rivalled the species as the ecologist's principal interest and gradually the rich new domain of theoretical population ecology arose. Population ecologists turned to experiment and to mathematical refinement, but faced the charge that their premises represented serious oversimplification and their empirical studies ignored the complexity to be found in the field.

The 1930s announced the ecosystem concept, but that idea came into its own only after the Second World War. Joined by systems theory and enthusiastically supported by the United States Atomic Energy Commission and other agencies, ecosystem ecology became the favoured child of the post-war years. Its promise of efficiency, holistic thinking and ready applicability added to its attractions. In an unparalleled crescendo of organization and expenditure, ecosystem ecology emerged as the keystone of the International Biological Programme, ecology's debut in Big Science. McIntosh's account of these developments is both comprehensive and droll; he has a keen eye for excesses as well as for science and scientists.



One of the fundamental problems in writing the history of ecology is the fact, itself not at all unexpected, that few new trends or interpretations displaced previous views and practices. The science persists and advances in a constantly unsettled condition. There has been and remains great dispute over the basic unit or units of ecological discourse — individuals, species, populations, communities, ecosystems or still larger entities; there is disagreement over the existence, character or relative importance of various ecological generalizations — succession, biogeochemical cycling, competitive exclusion; there is now available a host of research methods, undue allegiance to any one which might, as is always the case in the sciences, commit the investigator to one interpretive strategy and exclude other, perhaps promising possibilities. As noted, McIntosh has not elected to develop his history along these thematic lines.

One should attempt to do so, however, for what is needed today, in addition to detailed chronological accounts such as that offered here, is equally detailed analysis of several questions in the history of ecology. We need to know, for example, how and why animal ecology followed a course different from that of plant ecology; why population phenomena rose to the fore during the 1920s and 1930s; what role, if any, ecology played in the evolutionary modern synthesis; what have been the advantages of large-scale scientific research; and what have been the factors behind the shifting emphasis upon cooperative and competitive ecological models. We still need, despite the flood of literature seemingly directed to the topic, a clear view of the relationship of ecology as an aspiring natural science and the environmental movement as a political, social and moral campaign. The historian's obligation is to address such matters and not to rest content with a chronicle of events.

The Background of Ecology offers excellent preparation for such study. The author omits very little in the Anglo-American tradition — Continental ecology was not part of McIntosh's brief — and provides an up-to-date and virtually exhaustive bibliography of relevant primary and secondary materials. Moreover, his classification of doctrinal tendencies and portrayal of leading controversies provides a valuable guide to a vast and still largely uncharted sea. Regrettably, only within certain of its sections is McIntosh's volume a work of incisive interpretation, but it is throughout a reference work of distinction and one which is sure to be of interest to ecologists as well as to historians. □

William Coleman is Dickson-Bascom Professor in the Humanities and Professor of History of Science and History of Medicine at the University of Wisconsin, Madison, Wisconsin 53706, USA.

Private life of radiochemistry

Alwyn M'Kay

George de Hevesy: Life and Work. By Hilde Levi. *Rhodos International, Copenhagen/Adam Hilger: 1985. Pp.147. Dkr.120, £15, \$25.*

GEORGE de Hevesy (1885–1966) was one of the great pioneers of radiochemistry, perhaps the greatest. He invented many of the principal radiotracer techniques, for which he was awarded the 1943 Nobel Prize for chemistry, and the radiochemical textbook he wrote jointly with Fritz Paneth was for years the standard work on the subject. Though a chemist, he has been called “the father of nuclear medicine”, since it was to a large extent through his efforts that this field was opened up.

There is, however, surprisingly little in print about the man himself. There are three obituaries, one based almost entirely on his autobiographical notes, but not much else. It is fortunate that his long-serving assistant has given us this book.

Hevesy emerges as an enigmatic figure. He was aristocratic, courteous, reserved; one seldom talked to him about anything but science and its affairs. He took immense pleasure in his work, which is on record for all to see, but revealed little of his inner self, even in his letters to his closest friends. Life is more than science, yet Levi has been able to record no word of Hevesy's on religion or art, hardly anything on his family, and surprisingly little on political affairs considering that Nazism forced him to move from Germany to Denmark in 1934, and then to Sweden in 1943. Having a Hungarian passport, he could make these journeys freely despite being Jewish.

The book provides some illuminating sidelights on Niels Bohr, in whose Institute Hevesy spent two long and fruitful periods (1920–1926 and 1934–1943), at Bohr's personal invitation. Bohr admired Hevesy's abilities, which were very different from his own, and eagerly encouraged him to launch into the almost uncharted area of radiobiology in the second of these periods, arranging for Levi to become his assistant. She naturally describes the resultant scientific achievements in some detail, from first-hand knowledge.

Hevesy's earlier applications of radio-tracers to physicochemical problems, starting from work under Rutherford in 1911 and 1912 in Manchester, receive less attention. So long as the only tracers available were the natural radioelements, the only quantities he could measure, such as the solubility of lead chromate, were of limited interest in themselves. There was, however, great originality in his physicochemical ideas, which laid the foundations of the tremendous later developments in the use of radiotracers.

The author's command of English is generally excellent, but in places the text would have benefited from the attentions of a sub-editor. A particular point which may mislead is the use of the phrase “first of all”, not in a temporal sense, but to mean “above all” or “primarily”. Such small blemishes apart, the book tells its story well. Scientific matters are lucidly described (though the layman may find certain passages difficult even with the aid of the glossary provided) while the biographical material will be of interest to anyone concerned with the history of physics and chemistry in the twentieth century. □

Alwyn M'Kay, Thurso, 40 Lockstile Way, Goring, Reading RG8 0AL, UK, was formerly a Group Leader at the Atomic Energy Research Establishment, Harwell. He worked with Hevesy in Copenhagen between 1935 and 1937.



Meeting of minds — Haber's visit to Copenhagen in 1921. Standing: Neils Bohr, E. Güntelberg and I. Brønsted. Seated: G. Hevesy, Mrs A. Delbanco and Fritz Haber

Getting to grips with chaos

Michael Berry

Deterministic Chaos: An Introduction. By Heinz Georg Schuster. *Physik-Verlag*, 1985. Pp.220. DM98, \$43.75.

IN THE more fully developed sciences, natural laws are expressed as mathematical equations describing how the development of a system is uniquely related to ("determined by") its state at some other, usually earlier, time. (Quantum mechanics is no exception: its celebrated indeterminacy restricts what may be measured in any given state, not the way in which the state evolves.) Determinism does not imply predictability, however, and indeed a wide variety of systems that evolve causally, with no source of external noise, exhibit irregular and even random behaviour. The common property of deterministic chaotic systems is sensitive dependence on initial conditions: as a result of instability in the dynamical equations, two systems initially in very similar states will soon evolve to widely separated ones.

This is not a new idea. Maxwell understood it more than a century ago, and, shortly afterwards, Poincaré began to develop much of the appropriate mathematics. What is new is the emergence of "chaology" as the subject of intensive study in a variety of areas using a variety of techniques. The areas of application range from celestial mechanics (gaps in the asteroid belt) and fluid turbulence to physiological arrhythmia (heart attacks and cot deaths) and the populations in successive generations of an ecosystem. The techniques range from physical experimentation through computer simulation to the purest mathematics.

In his book, Schuster gives a very useful summary of the main ideas of the subject as it now stands. Although a physicist by training and style, he organizes his treatment by the logic of the mathematics, which is based on the concept of a dynamical system. This is any set of equations, describing how a set of variables changes with time, for which a uniqueness theorem guarantees that for each specification of the variables at one time (initial state) there is just one set of values at a later time (final state). This is a very broad framework: "time" may be continuous (as in mechanics, classical or quantum) or

discrete (as in the successive generations of an ecosystem); the set of variables may be finite (like the coordinates and velocities of a finite collection of particles) or infinite (like the triplet of velocity components at each position in a fluid).

Most of the book is devoted to simple mappings, where dynamics is reduced to a rule describing how a point in a low-dimensional space (a plane or even a line segment) jumps from "time" n to "time" $n+1$. This is a sensible strategy because much more complicated systems (such as turbulent fluids) appear to have, embedded in them, mappings which organize much of their behaviour. And astonishing complexity can indeed arise from multiple repetition of a simple map. Unraveling this has led to new concepts of wide applicability — strange attractors, Feigenbaum sequences, Lyapunov exponents, Kolmogorov entropy, for example. These and more are explained with maximum

clarity and the minimum (but subtle) mathematics; abundant illustrations, including beautiful colour plates, also help the reader along.

When there is no friction (a useful idealization in celestial mechanics and particle accelerators) different concepts apply. These are well summarized but, as the author admits, his treatment here is less original because many other accounts of this topic are available. Finally, the question of chaotic behaviour in quantum systems is discussed, with the caution appropriate to the unsettled state of the subject.

Students about to begin research into chaos, and practising scientists new to the subject, will find this a book well worth reading. □

Michael Berry is Professor of Theoretical Physics in the H.H. Wills Physics Laboratory, University of Bristol, Tyndall Avenue, Bristol BS8 1TZ, UK.

Olfactory work

D. Michael Stoddart

Social Odours in Mammals, Vols 1 and 2. Edited by Richard E. Brown and David W. Macdonald. *Clarendon*, 1985. Pp.882. Vol. 1 £45, \$60; Vol. 2 £32, \$45.

It is difficult to know whether this work is a review or a textbook — it has some of the characteristics of both. The editors say they were prompted to gather together contributions from their band of 13 authors because

the growth of theoretically exciting ideas concerning social odours, the scattered publications and expertise [gave us] the feeling that a systematic review of the whole class could provide a realistic perspective and a useful reference work.

Perhaps if they had clarified their objectives rather more they would have decided on what exactly they wanted. In my opinion *Social Odours in Mammals* is, if anything, a textbook cum reference work, although very different styles are adopted by each author. Its lack of success as a review rests largely on the poor coverage of the literature published during the current decade. Scanning the 20 bibliographies to be found at the end of each chapter one sees very few references post-1981, and most of the 1983 and 1984 references (I found only one of the latter) are to papers written by the chapter authors themselves. Book production is a notoriously slow business, but the price for delay has here been high. Olfactory biology is a rapidly developing field in which an effective review must be up to date.

The textbook quality stems from the books' rigorous taxonomic approach to the essentially functional set of phenomena referred to in the title. It is a great pity

there is no subject index (the four indexes included — author, common names, Linnean names and odour sources — occupy almost 50 pages), for there is no way, other than by reading the whole work, to find the latest ideas on, say, scent marking, or species or individual recognition. And no quick way of discovering if advances in specific areas of rodent olfactory biology, to which much space is devoted, are paralleled in other groups. This is an omission which substantially mars the books' usefulness.

What these two volumes do provide is a series of articles examining all that is known about the role of odours in mammals. The rodents are dealt with in four separate chapters — one on the effects of odour on reproductive physiology, one on the *Myomorpha*, one of the *Sciuromorpha* and one on the *Hystrichimorpha*. The primitive eutherians and the primates are given a different treatment: a whole chapter is devoted to a case study of the tree shrew, for example, and a chapter each to the saddle-back tamarin and to human beings. Special treatment for these and not for other well-worked species, such as the golden hamster and the Norway rat, seems inconsistent. Nonetheless, the case-histories provide excellent overviews of the work of two or three leading laboratories in the field. I also particularly enjoyed the chapters on marine mammals and the edentates and pholidotes, as these groups are seldom covered. In all chapters the treatment is thorough, and many tables enhance the presentation.

As an initiate in the field of olfactory biology I find this a useful, if somewhat eclectic, reference work. But I will direct my students to other works to gain a more rounded and up to date impression of the subject. □

D. Michael Stoddart is Professor in the Department of Zoology, University of Tasmania, Box 252C, GPO Hobart, Tasmania, Australia 7001.

The classic anatomist's textbook *The Development of the Vertebrate Skull* by Gavin R. deBeer — first published in 1937 — has just been reprinted with a new Foreword written by Brian K. Hall and James Hanken. The book is published by the University of Chicago Press. £18.95, \$22.

Build-up around the planet

John Noble Wilford

The Space Station: An Idea Whose Time Has Come. Edited by Theodore R. Simpson. *Institute of Electrical and Electronics Engineers, New York/STM Distribution, Enterprise House, Ashford Road, Ashford, Middlesex TW15 1XB, UK:1985. Pp.295. \$19.95, £18.*

COMING to terms with the proposed American space station in any rational way is little short of impossible, if only because of what it is not. Unlike the Apollo Project, it is not a programme with a specific destination (the Moon) or a clear political objective (a bold challenge to Soviet leadership in space). Unlike the Space shuttle, it lacks the coherence of a single piece of hardware already agreed on and with a single function, which was to make travel to space more routine and perhaps less expensive. (No matter that this latter objective has yet to be realized; it was the project's decisive selling point.) What, then, can be said of or for the space station?

In directing the National Aeronautics and Space Administration (NASA) "to develop a permanently manned space station, and do it within a decade", President Reagan in 1984 offered few details of the station's architecture, capability or purpose. He spoke only of following "our dreams to distant stars, living and working in space for peaceful, economic and scientific gain". And even though NASA had lobbied fervently for the project, its officials had no specific engineering plans ready for carrying out the Presidential order. One of their first acts was to issue contracts to industry to develop space station designs and to identify who might need or want to use such a facility. Nearly two years after President Reagan's speech, the idea has yet to be translated into firm plans.

This much can be said: the space station is supposed to be a large facility in a near-equatorial Earth orbit, about 300 km above the surface. It would be composed of several units, including a control centre and a module for housing crews or as many as ten people. These components would be launched separately from the Space Shuttle. The facility would grow module by module to meet demand for laboratories for microgravity research, materials-processing "factories", remote-sensing platforms and staging bases for missions to more distant orbits and the planets.

Some of the visions and engineering particulars for such a facility are described in *The Space Station*, which is a collection of articles by NASA officials, politicians,

science policy makers and engineers. Although the prevailing view is favourable to the principle of the space-station, the book cannot be dismissed as a propaganda tract. The editor, Theodore R. Simpson, an experienced aerospace engineer, has included several valuable discussions about the history and process of American decision-making in space matters. Several of the contributors are sceptical of the idea.

For those who want a preview of what the orbiting facility might be, two NASA engineers offer a comprehensive discussion of various designs that have been considered. In the book's illustrations, the station is usually characterized as a cluster of cylindrical modules, docking ports for shuttles and the long arms of solar-power panels. Romantics will be disappointed, for nothing resembles the toroidal station in the movie *2001: A Space Odyssey*. But as other contributors, from the Congressional Office of Technology Assessment, point out, "We would be surprised if NASA's present space station plans were carried through to completion without major change". They suggest that, in the absence of clear-cut goals, "it is simply not yet possible to judge, objectively, whether or not NASA's presently suggested infrastructure elements are truly appropriate and worth their substantial cost".

Similar reservations are expressed throughout the book. The Pentagon's lack of enthusiasm, reiterated in one chapter, must be of particular concern to space station advocates; Pentagon backing is usually essential to big-money space efforts.

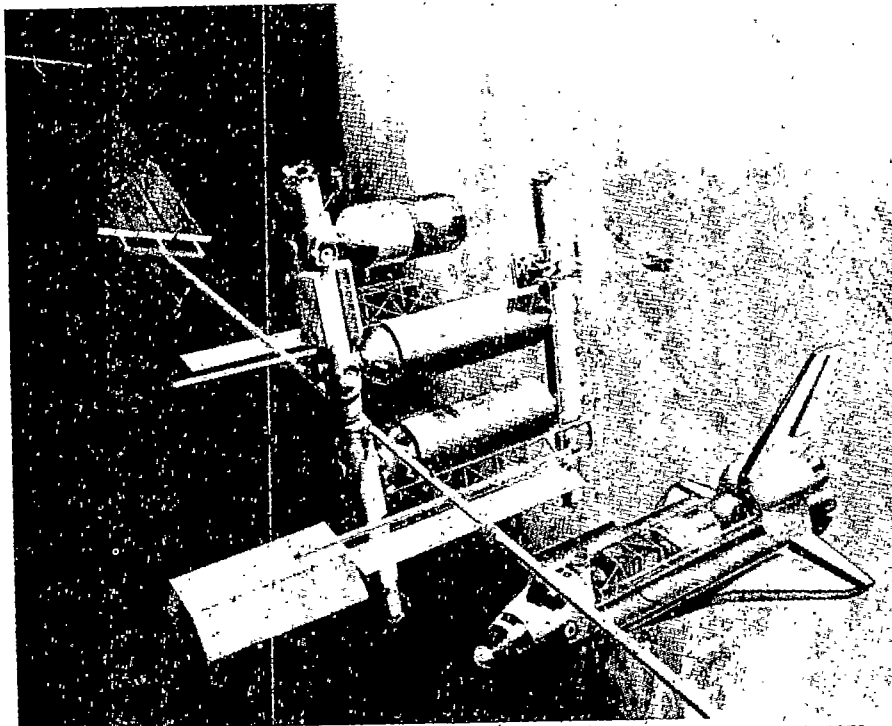
Thomas M. Donahue, a physicist at the University of Michigan, who heads the Space Science Board of the National Academy of Sciences, states the opposi-

tion of many scientists to the idea. They doubt that a space station would add significantly to what they can already accomplish with the shuttle and automated satellites. Moreover, they fear that the station's costs will drain money from their own cherished projects. But Dr Donahue seems resigned to the station's inevitability: "We hold that the decision to build a space station will be made for reasons other than its usefulness to space science and applications".

Victor M. Reis, a former science policy adviser in the Reagan Administration, takes this point even further. He acknowledges that each and every one of the projected space station missions could be done well by a judicious combination of shuttles, Spacelabs, automated platforms and satellites. Nonetheless, he argues that there is one "powerful argument" in support of the project, which is, in effect, to assure NASA of a robust future. Mr Reis is probably correct in calling this "the fundamental issue" of the space station decision. But he cautions that a decision to go forward with the project would be justified only if "it can be done in harmony with NASA's other missions and responsibilities". This will not be easy, he notes, because NASA's big manned programmes have a way of consuming the lion's share of NASA's budgets.

The Space Station will not be satisfying to those who seek to win converts to the space-station cause or to those who are its outright opponents. More than most of the authors may have intended, the book reveals why the project has so far had such a cool to mixed reception. □

John Noble Wilford is a reporter on science and space for the New York Times.



Not yet a reality — the concept of a Space Operations Center developed by NASA in 1979.

Sociological problems of high-energy physics

from Andrew R. Pickering and W. Peter Trower

Large-scale collaborative projects between experimental high-energy physicists have produced sociological problems that will need thought to resolve.

TEAM research entered the study of elementary particles in the immediate aftermath of the Second World War. The collaborative methods which had borne fruit in the Manhattan Project were carried over into the new speciality of high-energy physics. The most visible early manifestation of this organizational transfer was at the Radiation Laboratory of the University of California, Berkeley, where E.O. Lawrence's pre-war development of the cyclotron had led to a central role in atomic weapons research¹. As R.R. Wilson, the first director of the Fermi National Accelerator Laboratory, recalled in 1970, "A word was even coined — Berkeleitis — to describe the syndrome that existed there"².

Despite the misgivings of physicists who harked back to the pre-war days of individualistic research, Berkeleitis soon became endemic to high-energy physics, growing more rampant with the passing years³. Until the mid-1960s it was still possible for a small group of, say, less than ten physicists to make a significant contribution to high-energy physics; by the late 1970s the complement of a typical collaboration had grown to around 50. Today, groups of around 200 physicists are assembling in preparation for experiments at Europe's next new big machine — the Large Electron-Positron Collider (LEP), due to come into operation at CERN, Geneva, in 1987.

As collaboration size has grown, so too has the duration of typical experiments. In the 1960s an experiment could be mounted and data analysed within a period of months, while current experiments can span more than a decade from first conception to final publication of results. Thus, in terms of both personnel and duration, high-energy physics experiments have moved ever further from the pre-war stereotype of the lone researcher at the laboratory bench, and have come more to resemble large-scale engineering projects.

Trends

The underlying trend towards larger collaborations and extended timescales in experimental high-energy physics is not hard to understand. The relatively generous funding which high-energy physics has enjoyed throughout its history has made possible the construction of a succession of particle accelerators of ever-increasing size, and sophisticated experimental techniques have been developed to exploit the

high-energy beams thus made available. Instead of a single detector, a modern experiment deploys a complex multi-element array, costing millions of dollars and requiring an army of specialists to design, build and run. Considerable resources are also required for subsequent data analysis.

Neither is it hard to appreciate the benefits of this form of research. The discoveries at CERN of the electroweak intermediate vector bosons, W^\pm and Z^0 , made by two groups totalling nearly 200 physicists, were the culmination of a decade of rapid progress in understanding elementary-particle interactions. But such benefits have been bought at a price. Our aim here is to outline the sociological problems which are the symptoms of Berkeleitis. Our discussion is organized around twin themes: the frustration of individual initiative and creativity within large collaborations, and the consequent tendency to conservatism and orthodoxy in communal practice. (The frustrations inherent in team research have been impressed upon us in informal communication with many experimenters. It seems clear that the present trend towards non-accelerator experiments (for example, searches for free quarks, magnetic monopoles, proton-decay) is, in part, a manifestation of these frustrations.) We will show that the problems arising within individual experiments spill over into programmes of experiment. They are reinforced by the institutional structure of experimental high-energy physics, and have been further exacerbated by conceptual developments in the field over the past decade. Finally, we ask whether anything can be done to check current trends to gigantism and orthodoxy in high-energy physics research.

The rationale for team research is the division of labour. A large collaboration comprises several groups of physicists, each drawn from a single university or laboratory. In setting up and running an experiment, each group takes on responsibility for particular elements of the overall apparatus. Data analysis is likewise broken down into component tasks allotted to different groups. Thus, along with the division of labour goes a degree of specialization beyond that normally associated with scientific research.

This is where sociological problems begin to arise, particularly for junior physicists — postgraduate students and young postdoctoral researchers. Even within a

single group of experimenters, research tasks are subdivided. And junior physicists are usually those responsible for the basic items of hardware and software. They thus acquire detailed knowledge of only one small part of an enormous and diversified project, and their chances of making a significant contribution to the overall course of the experiment outside their area of expertise are slight.

Managers

Senior physicists encounter a similar problem, but for different reasons. They oversee and coordinate the work of a group or groups, and are thus obliged to take on administrative responsibilities more familiar to managers of large technological enterprises. Another function of senior physicists is to secure the material resources necessary for the success of the experiment, and to negotiate with laboratory managements for beam-time, workspace and so on. Here they function as entrepreneurs. Again, even at the most senior level, the role of creative scientist can be submerged for long periods of time (if not forever) by the multifaceted organizational demands of a large collaboration.

In a variety of ways, then, many physicists find their creative ambitions and aspirations frustrated in the course of high-energy physics experiments. And, all things being equal, opportunities for innovation decrease in proportion to the degree of specialization and the associated administrative load — that is, in proportion to collaboration size and the duration of the experiment.

Only in a field where long experiments were commonplace, though, would one even look for creative opportunities within the development of a single experiment. The classic locus for creativity in experimental science lies in the gaps between experiments: in the conception of new techniques or new problems to tackle. But here too Berkeleitis has a deadening effect. One can discern at least two sources of conservatism in experimental design in high-energy physics. The first is that specialization is self-reproducing. As discussed above, in his research training, the young physicist acquires a narrow, specialized competence in some limited aspect of experimental high-energy physics. This competence then becomes his most precious asset in the pursuit of a research career, so valuable (to himself and to others) that opportunities for future variety in research practice are highly

circumscribed. And the upshot of this is an inhibition of technical innovation within high-energy physics experiment as a whole.

Since techniques are usually appropriate to the exploration of a limited class of phenomena, technological conservatism itself implies conservatism in the choice of research problems, and both forms of conservatism are reinforced by a second source: the fear of failure. The senior physicist, or physicists, who propose to conduct a contemporary high-energy physics experiment risk not only their personal reputations, but also the efforts and careers of the many less senior physicists who will be involved in the project. If the experiment fails — if it does not produce interesting data or, indeed, any data at all — hundreds, possibly thousands, of man-years of effort and millions of dollars will have been wasted. Not surprisingly, therefore, there is a tendency to conservatism in experimental programmes, with new experiments aiming to investigate phenomena of well-established interest using well-established techniques.

Rewards

So far we have outlined the obstacles to initiative and creativity implicit in collaborative research of long duration: the twin requirements of specialization (with its self-reproducing character) and administration, and the ever-present risk of failure. In themselves, the impact of these factors can be overestimated. Against them must be set the potential rewards for successful innovation — symbolic rewards like Nobel prizes, and material rewards such as career advancement. There are, however, institutional factors in high-energy physics which serve to structure even successful innovations, to foster a limited set of technological developments or problem choices at the expense of others. This, too, is a form of conservatism, which again acts to stifle individual initiative.

The origins of institutional conservatism in high-energy physics lie in the centralization of research resources. Despite generous funding, the expense of building and running particle accelerators has long been beyond the resources available to individual universities. Over the history of the field, the facilities for high-energy physics experiments have been gathered together into a handful of regional, national and international laboratories at which collaborations assemble to perform their experiments before departing to their home universities for data analysis.

Together with the centralization of research resources has gone a proliferation of committees. Access to experimental beams and funding for experiments are controlled by committee, likewise planning and funding for new facilities — new accelerators or new major items of experimental equipment. Thus any potential innovator is faced with an institutional

hurdle. The typical disposition of committees towards conservatism is well known, and the experimenter who does not propose to tackle an established problem with established techniques is likely to find his proposal rejected.

As Nobel Laureate Luis Alvarez put it: "Our present scheduling procedures almost guarantee that nothing unexpected can be found". As it happens, there is a twofold irony to this quotation. In his leadership of the 72-inch bubble chamber programme at Lawrence's Radiation Laboratory, Alvarez did more than any other to demonstrate the benefits of Berkeleyitis in experimental high-energy physics⁵. (For a discussion of the Alvarez group, see ref. 6.) And at the time when he wrote, 1973, high-energy physics was characterized by a pluralism of research strategies that was soon to vanish.

The 1970s saw the rapid development of the 'new physics' world-view in high-energy physics, in which the world was seen to be built from quarks and leptons interacting according to the dictates of twin gauge theories — quantum chromodynamics (for the strong interaction) and the Weinberg–Salam–Glashow unified electroweak theory. And the sociological correlate of the 'new physics' was the 'new orthodoxy' (as it was christened by high-energy physics theorist James Bjorken)⁷. Within the new orthodoxy, communal research practice in high-energy physics became almost exclusively organized around the new-physics world-view. The effect was most striking in the experimental fields, where the institutional committee structure reflected the majority view and effectively enforced the new-physics dominance of research. Since the late 1970s it has been difficult, if not impossible, to mount a high-energy physics experiment which does not promise to engage directly with the interests of gauge theorists.

One consequence, then, of the rise of the new orthodoxy has been a close circumscription of opportunities for initiative in the choice of topics for experimental investigation. Along with this restriction on problem choice have gone restrictions on acceptable forms of technical innovation. As currently elaborated, gauge theory offers an analysis of only a limited range of rare phenomena. Investigation of those phenomena (in the presence of an overwhelming background of 'uninteresting' processes) requires large, complex experiments and highly sophisticated electronics — precisely the experiments which call for large collaborations working together over a period of years. Larger and larger teams have assembled to man the ever-larger experiments expected to probe the fine details of already rare phenomena, leading to the projection of 200 member teams for LEP.

Thus, more than any other factor, it has been the new physics (via the new orthodoxy and the institutional structure of

high-energy physics) which has driven the trend to gigantism in experimental physics throughout the 1970s and into the 1980s. And, as an unintended consequence, it has multiplied all of the sociological problems discussed above. The degree of specialization in the experimental physics community, the extent of administrative responsibilities and the risks attached to failure have all increased with the coming of the new physics. Opportunities for initiative and creativity have decreased in proportion.

The new physics, then, through the grip of the new orthodoxy on experimental high-energy physics, is experienced by many physicists as making the life of the researcher more frustrating and less rewarding. What of the future? At present, the new orthodoxy, with its attendant drive to gigantism and the multiplication of sociological problems, is set to reproduce itself indefinitely. Through the institutional structure of high-energy physics, the future as well as the present of experimental research has been given over to the new physics. The next generation of big machines — at which the next generation of experimenters will learn their trade — are explicitly conceived as new-physics facilities. LEP, for example, is intended to operate as an intermediate vector boson factory (see, for example, ref. 8).

The state of high-energy physics is therefore not entirely satisfactory, despite the conceptual triumphs of the new physics. The existence of a self-perpetuating orthodoxy is anathema to many physicists, especially an orthodoxy which multiplies pre-existing sociological problems by further stifling opportunities for individual initiative. The question arises: can anything be done to ameliorate this situation? Clearly, a return to the pre-war days of the lone researcher is impossible — Berkeleyitis is intrinsic to the technical and institutional fabric of experimental high-energy physics — but can the slide into gigantism be checked (for those who wish it)?

Here there is a straightforward suggestion. The institutions of high-energy physics should be used to relax rather than to enforce the stranglehold of the new physics upon experiment. A fraction of the available resources should be set aside for those who would, for one reason or another, follow a heterodox path. In this way, the oppressive aspect of the current orthodoxy could be *de facto* eradicated. And, since it is the new-physics emphasis on rare phenomena which is driving the present increase in collaboration size and experiment duration, the route would be open for individualistically inclined researchers to move towards their chosen form of practice. Unconventional paths of inquiry could be followed, novel small-scale experimental techniques could be developed and old techniques rescued from oblivion, all with unpredictable but possibly major consequences for future

patterns of research.

It is important to stress that the argument is not that the new-physics world-view should be abandoned — its virtues are too well established for that — but simply that some opportunities should be left for research outside the orthodoxy. (It is worth recalling that it was precisely such heterodox research that led to the 1974 discovery of the J-psi particle and the establishment of charm — an episode that marked a watershed in the development of the new physics.) Of course, in comparison with a well-established orthodoxy, heterodox experimental proposals inevitably appear lightweight — perhaps frivolous or even incomprehensible — and committees find it correspondingly hard to support them. But this point has already been adequately dealt with by the mathematical physicist Freeman Dyson, who suggests that funding agencies should allot somewhere in the region of 10–25 per cent of their resources to heterodox research. (The figure of 10 per cent is also suggested by Muller. Alvarez argues that proposals in experimental high-energy

physics should be assessed solely on the basis of the experimenters' past performance, and without regard to consensually perceived theoretical significance.)⁹

It should be noted that although this article has focused upon sociological problems arising in experimental high-energy physics, problems also exist in theory. Here again institutional structures presently act to discourage practice outside the gauge-theory orthodoxy. Dyson's article is, in fact, concerned with encouraging theoretical rather than experimental diversity. His conclusion is, however, relevant to both theoretical and experimental practice: "We should not be afraid of looking foolish or even crazy. We should not be afraid of supporting risky ventures which may fail totally . . . Organizations which only support research where there is no chance of mistakes will in the end support only mediocrity. If we proceed with good sense and courage to support unfashionable people doing things that orthodox opinion considers irrelevant or crazy, there is a good chance that we shall rescue for science . . . people

whose ideas will still be famous long after all our contemporary fashionable excitements are forgotten".

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ARTICLES

Concentration of methane in the troposphere deduced from 1951 infrared solar spectra

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The 1–2% increase in tropospheric methane determined from gas chromatographic measurements since 1977 has important implications for atmospheric photochemistry and climate. To extend the measurement baseline, we have analysed solar absorption spectra and determined mean troposphere CH₄ mixing ratios for the years 1951 and 1981, and find an average rate of CH₄ increase of 1.1 ± 0.2% per year for this period.

ALTHOUGH methane is only a trace atmospheric constituent at the part per million by volume (p.p.m.v.) level, it has a significant impact on the photochemistry of both the troposphere and stratosphere and on the Earth's climate. In the troposphere, the methane oxidation chain initiated by the reaction of CH₄ with the hydroxyl radical (OH) leads to significant photochemical production of H₂, CO and O₃ (ref. 1). In the stratosphere, CH₄ is a source of odd hydrogen species (HO_x) which catalytically destroy O₃ (ref. 2). In addition, CH₄ reacts with atomic chlorine in the stratosphere, terminating the odd chlorine cycle, which catalytically destroys O₃ (ref. 2). Methane also contributes to the atmospheric greenhouse effect by its absorption in the 7–14-μm spectral region³.

Over the past 5 years, several studies have reported that levels of CH₄ in the troposphere may be increasing. The analysis of urban or suburban total hydrocarbon data in New Jersey over 10 years (1968–1977) led to the suggestion that CH₄ (which was not monitored explicitly, but is the major component of the measured total hydrocarbon concentration) may have increased at the rate of ~0.6% yr⁻¹ (ref. 4). Analysis of almost 1,600 nearly continuous measurements of CH₄ obtained over a 22-month period (January 1979–October 1980) at Cape Meares, Oregon, indicated that CH₄ was increasing at a rate of 1.9 ± 0.5% yr⁻¹ (ref. 5). A similar increase was found in measurements over

western Europe from 1977 to 1981 (ref. 6). Measurements of CH₄ between November 1977 and November 1980 in remote locations between 55° N and 53° S indicate a 1% yr⁻¹ increase in CH₄ between January 1978 and January 1980 (ref. 7). Additional Cape Meares measurements obtained between October 1980 and December 1981, as well as 3 yr of measurements from the Arctic Circle to the South Pole, suggest a global increase of 1–1.9% yr⁻¹ (ref. 8). The results of >800 CH₄ measurements over southern Australia (September 1980–March 1983), from the surface to the upper troposphere, show an average rate of tropospheric CH₄ increase of 1.3% yr⁻¹ at December 1981 (ref. 9). Three different sets of CH₄ measurements were examined for trends by Ehhalt *et al.*¹⁰: (1) 49 tropospheric profiles obtained from 1965 to 1967 and 1971 to 1974 by aircraft flights at the National Center for Atmospheric Research (NCAR); (2) measurements obtained from 1968 to 1979 from ships and aircraft by the Naval Research Laboratory (NRL); and (3) published measurements of the total atmospheric CH₄ column amount deduced from ground-based infrared spectra recorded between 1948 and 1975. Ehhalt *et al.*¹⁰ summarized their conclusions from these three data sets, as well as the other measurements of CH₄, in the following way: (1) There was little or no increase in CH₄ between 1948 and 1965, shown by published analyses of the ground-based infrared spectra. (2) NCAR

and NRL measurements and the data of Graedel and McRae⁴ indicate a $0.5\% \text{ yr}^{-1}$ increase in CH_4 between 1965 and 1975. (3) There is good evidence for a global CH_4 increase of $\sim 1\text{--}2\% \text{ yr}^{-1}$ between 1978 and 1980 based on several sets of measurements⁵⁻⁸.

Ehhalt *et al.*¹⁰ also discuss the analyses of air trapped in Greenland and Antarctic ice from 100 to 2,500 yr ago, which yield significantly lower levels of CH_4 (refs 11-13). Ehhalt *et al.*¹⁰ suggest that these low CH_4 levels are caused either by lower levels of atmospheric CH_4 at the time the air was trapped in the ice or by trapped CH_4 that may have been lost by diffusion through the ice. They conclude that the relation of CH_4 levels in ice to the atmospheric CH_4 levels of the past remains unclear.

Although the published ground-based infrared spectral measurements indicate little or no increase in CH_4 in the troposphere between 1948 and 1965 (ref. 10), these early studies were limited in their accuracies by uncertainties in the assumed values for the spectroscopic line parameters, the vertical CH_4 profile shape, the vertical pressure-temperature profile and the relatively simple methods of analysis available at that time. To obtain a more accurate determination of the early CH_4 tropospheric concentration, we initiated a study to deduce the levels of tropospheric CH_4 and other gases from such historical spectra.

Here we report an analysis of infrared solar absorption spectra recorded in April 1951 at the Sphinx observatory of the Jungfraujoch International Scientific Station in the Swiss Alps (latitude 46.5° N , longitude 8.0° E ; elevation 3,578 m)¹⁴. We have used the best available line parameters for CH_4 and interfering lines of H_2O in the analysis, and determined the average tropospheric mixing ratio of CH_4 from a line-by-line, multi-layered atmospheric, nonlinear least-squares spectral fitting procedure. Correlative radiosonde and surface pressure-temperature measurements were used to determine the vertical pressure-temperature profile for the analysis. A mean tropospheric mixing ratio of $1.14 \pm 0.08 \text{ p.p.m.v.}$ has been deduced for CH_4 from the analysis. As uncertainties in line parameters remain a problem in interpreting spectroscopic data, we also derived the mean concentration of CH_4 in the troposphere from analysis of the same region in Kitt Peak solar spectra taken in February 1981. From the derived value of $1.58 \pm 0.09 \text{ p.p.m.v.}$, which is close to other modern determinations, and our value for April 1951, we determine an average rate of increase of $1.1 \pm 0.2\% \text{ yr}^{-1}$ for 1951-81.

Detailed spectral analysis is the key to deducing the concentrations of trace species in the troposphere from historic solar spectra. Hence, most of this paper deals with the spectral analysis that led to the determination of a significantly lower tropospheric concentration of CH_4 in 1951, and with the reconciliation of this new, lower value with the older, higher spectral-deduced values.

Observations and spectral analysis

The Jungfraujoch measurements selected for analysis were made with a coelostat and Pfund-type prism-grating spectrograph on the morning of 15 April 1951, between 09 h 59 min and 10 h 17 min UT. These data cover the $2,901\text{--}2,926\text{-cm}^{-1}$ spectral interval and have been published as part of an infrared photometric atlas¹⁴. According to Table 2 of the atlas, the zenith angle during the measurements decreased from 41.5 to 39.8° . The infrared signal was detected by a Perkin-Elmer thermocouple together with a 13 c.p.s. chopping system and amplifier manufactured by the same firm and connected to a Leeds and Northrup type-G Speedomax recorder. A grating with a $16 \times 12 \text{ cm}$ ruled surface and an effective slit width of 0.24 cm^{-1} was used in the region considered here. Additional details of the instrumentation are given in the atlas¹⁴ and elsewhere^{15,16}.

Absorption in the analysis interval is dominated by lines of CH_4 with minor contributions from water vapour and solar lines. The spectral data were digitized from the atlas using a Houston Instruments Hipad digitizer attached to a model PGM-2 computer (Superset). The zero-signal level indicated in the atlas has been assumed. The wavenumber scale of the digitized data has been calibrated based on measurements of the line centre posi-

Table 1 Some atmospheric parameters assumed in the analysis of Jungfraujoch spectra

Layer boundaries (km)	Temperature (K)	Pressure (mbar)	CH ₄ mixing ratio (p.p.m.v.)	
			Case A	Case B
3.578-5.0	255.9	594.0	1.60	1.60
5.0-7.5	243.2	456.2	1.60	1.60
7.5-9.0	228.1	339.0	1.60	1.60
9.0-12.0	216.7	244.8	1.60	1.60
12.0-15.0	215.6	152.4	1.55	1.55
15.0-18.0	216.2	95.1	1.43	1.43
18.0-20.0	216.2	63.7	1.25	1.32
20.0-22.0	216.3	46.7	1.10	1.26
22.0-24.0	216.7	34.2	1.00	1.20
24.0-26.0	219.0	25.0	0.90	1.10
26.0-28.0	222.8	18.4	0.83	1.00
28.0-30.0	226.7	13.6	0.75	0.90
30.0-32.0	231.9	10.1	0.67	0.80
32.0-35.0	240.1	7.13	0.58	0.70
35.0-40.0	252.4	4.25	0.45	0.60
40.0-60.0	265.2	1.54	0.25	0.38
60.0-100.0	233.6	0.10	0.05	0.05

Total vertical column amount of air molecules $1.380 \times 10^{25} \text{ cm}^{-2}$. Altitude of the tropopause $\approx 10.2 \text{ km}$.

tions of 13 absorption features distributed nearly evenly in wavenumber throughout the region. The centre positions were obtained with a line-finding algorithm similar to that used in a recent analysis of CO_2 laboratory spectra¹⁷. For unblended lines, the wavenumbers from the 1982 Air Force Geophysics Laboratory (AFGL) major gas compilations¹⁸ were adopted. For blended features, a strength-weighted mean position from the 1982 AFGL major gas compilation¹⁸ was adopted initially; this value was refined by measuring the line centre position of the feature in a preliminary least-squares best-fit calculated spectrum. Linear interpolation was used to determine the wavenumbers of data between the calibration points.

Three sources of meteorological information were used in the determination of the profile of pressure and temperature as a function of altitude above the Jungfraujoch station on 15 April 1951: (1) surface measurements of pressure (P) and temperature (T) at the Jungfraujoch station on 15 April, interpolated for the time the infrared spectrum was recorded (P. Hachler, personal communication); (2) tropospheric radiosonde P - T soundings from Payerne (46.5° N , 6.6° E) for 05 h UT on 14 April and 16 April (P. Hachler, personal communication); and (3) stratospheric-mesospheric climatological P - T profiles for April derived from a composite of 5-20 yr of daily radiosonde and satellite measurements for the mid-latitude Northern Hemisphere^{19,20}. The above information was used to define the P - T profile up to the upper mesosphere; above this altitude, mid-latitude standard atmosphere values for spring were assumed²¹.

The tropospheric portion of the P - T profile was derived by first interpolating the two Payerne radiosonde profiles to the time and date of the spectroscopic measurements. Not surprisingly, the interpolated values corresponding to the altitude of the Jungfraujoch station (650.5 mbar and -12° C) differed slightly from the surface measurements (655 mbar and -14° C). To improve the compatibility of these two sources of meteorological information, we adopted the measured surface values and adjusted slightly the altitudes corresponding to the interpolated upper air P - T measurements, using the hydrostatic equation. A standard meteorological thermodynamic diagram was used for this purpose.

The 17-layer model adopted in the analysis of the Jungfraujoch spectrum is presented in Table 1. The airmass per layer was calculated with the FSCATM ray-tracing program from FASCOD1C²², assuming the P - T profile discussed above and the mean solar zenith angle given for the observations¹⁴. The pressures and temperatures listed for each layer are effective values weighted by the density of air along the refracted atmospheric path.

Ground-based solar absorption spectra contain only very limited information about the altitude distribution of atmospheric gases. Therefore, we have assumed a relative mixing-ratio profile for both CH_4 and H_2O based on reported measurements. The absolute values are derived by scaling the relative mixing ratio in each layer by a single multiplicative factor determined for each gas from the spectroscopic analysis.

As no measurements of the vertical distribution of CH_4 were made in 1951, calculations have been performed assuming two profile shapes based on recent data (Table 1). For both cases, we have adopted a constant mixing ratio of 1.60 p.p.m.v. below 12 km. The stratospheric portion of the case A profile is representative of CH_4 measurements made between 40°N and 60°N using *in situ* and remote techniques, including the results of the NIMBUS 7 Stratospheric and Mesospheric Sounder (SAMS) instrument. It has been obtained by drawing a smooth curve through the values presented in Fig. 7 of ref. 23. The case B profile assumes higher mixing ratios in the upper stratosphere than were usually observed by NIMBUS 7 SAMS at 40°N – 60°N . It is representative of results from the same data sources for the 20°N – 40°N latitude range and has been derived in the same way from Fig. 6 of ref. 23. The case B values have also been used in the analysis of the 1981 solar spectra from Kitt Peak (32°N). The 1962 US Standard Atmosphere water vapour distribution²⁴ has been adopted for the H_2O profile shape. High-resolution ground-based spectra show lines of NO_2 in this spectral region^{25,26}. Although these lines are too weak to be identified in the Jungfraujoch spectra, we have included them in the radiative calculations assuming a published NO_2 reference profile²⁷.

Line parameters for CH_4 , H_2O and NO_2 were taken from the 1982 AFGL major and trace gas compilations^{18,28}. A T^{-n} temperature dependence was assumed for the air-broadened half-widths with values of $n=0.9$ for CH_4 , $n=0.5$ for H_2O and $n=0.92$ for NO_2 . The value for CH_4 is based on several sets of laboratory measurements^{29–31} that have found values of n near unity for self-, N_2 - and air-broadening. The value for NO_2 is taken from a recent study on the N_2 broadening of lines in the ν_3 and $\nu_2 + \nu_3 - \nu_2$ bands³². The value of $n=0.5$ for H_2O corresponds to the assumption of a temperature-independent collision diameter.

The analysis using the technique of nonlinear least-squares spectral curve fitting follows the procedures described recently for the interpretation of high-resolution ground-based spectra^{33,34}. This method has proven particularly suitable for the present study because of its accuracy in establishing the background level and its usefulness in regions of overlapping absorption lines, as observed in the Jungfraujoch data. In this study, a total of four parameters were adjusted simultaneously in the least-squares fittings to the digitized spectrum (point spacing = 0.02 cm^{-1}). Two of the parameters model the instrument response: one is for the background level of the spectrum and the other is used to fit the instrument line-shape function. Gaussian slit functions have been found for two representative grating spectrometers³⁵. We assumed this line shape in our analysis with the width of the gaussian function as an adjustable parameter in the least-squares fits. The other two parameters are the multiplicative scaling factors that convert the relative profiles of CH_4 and H_2O to absolute values.

Two sections of the digitized Jungfraujoch data were analysed assuming the case A profile shape for CH_4 . From the $2,903.0$ – $2,912.2\text{ cm}^{-1}$ region, a total vertical column amount of 1.510×10^{19} molecules cm^{-2} is deduced for CH_4 , corresponding to a tropospheric mixing ratio of 1.16 p.p.m.v. From the $2,915.2$ – $2,922.0\text{ cm}^{-1}$ region the corresponding values are 1.471×10^{19} molecules cm^{-2} and 1.13 p.p.m.v. The average of these two determinations of the CH_4 tropospheric mixing ratio (1.14 p.p.m.v.) is considerably lower than modern values^{5–9}. Fits with the case B CH_4 profile shape produced tropospheric CH_4 mixing ratios only 0.3% smaller than the case A values. The small magnitude of this difference results from the fact that only a small percentage of the total number of CH_4 molecules along

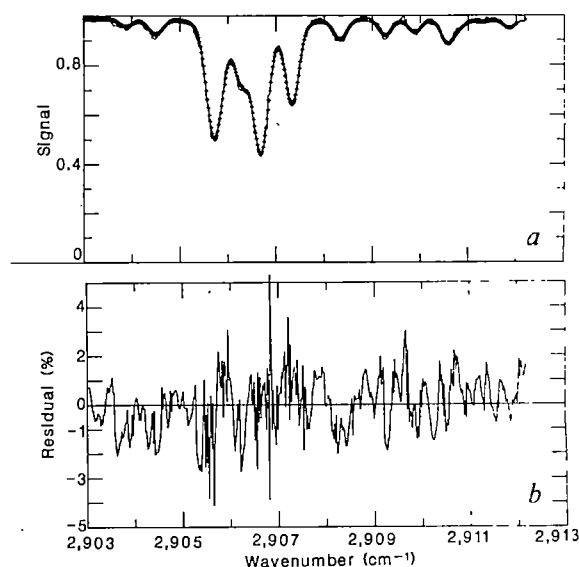


Fig. 1 Comparison (a) between the Jungfraujoch digitized spectrum (solid line) and a least-square best-fit to the data (plus symbols). Residuals (b) are observed – calculated values.

the atmospheric path are located in the middle and upper stratosphere where the two assumed profile shapes are different. Slightly larger changes in the tropospheric CH_4 mixing ratio were obtained by modifying the assumed profile shape in the lower stratosphere. For example, if the case A profile shape is assumed to be above 18 km and 1.60 p.p.m.v. is assumed for all altitudes below 18 km, tropospheric CH_4 mixing ratios 0.7% lower than the case A values are calculated.

As noted above, uncertainties in the assumed line parameters remain a major potential source of error in spectroscopic retrievals. Therefore, as a check on both our analysis procedure and the spectroscopic parameters that we have adopted, the CH_4 tropospheric mixing ratio has also been determined from modern data covering the same spectral region. For this purpose, we have selected a particularly dry spectrum recorded at an average solar zenith angle of 70.65° on 23 February 1981 from Kitt Peak (latitude 31.9°N , longitude 111.6°W ; elevation 2,095 m). The observations were made at 0.01 cm^{-1} resolution with the Fourier transform interferometer in the McMath solar telescope complex which is operated by the National Solar Observatory (see ref. 34 for details). To increase the validity of the comparison between the old and new data, the resolution of the Kitt Peak spectrum has been degraded by convolving the measured data with a normalized gaussian function having a width (cm^{-1}) equal to that retrieved from the Jungfraujoch spectral analysis. A correlative P – T profile based on radiosonde and global satellite measurements³⁶ has been assumed in the ray-tracing calculations. A tropopause height of 10.4 km is indicated from these results.

Assuming the case B relative mixing-ratio profile for CH_4 in the stratosphere (which is representative of measurements for the latitude of Kitt Peak), the total vertical column amount and concentration of CH_4 in the troposphere determined from the Kitt Peak spectra are 2.533×10^{19} molecules cm^{-2} and 1.56 p.p.m.v. from the $2,903.0$ – $2,912.2\text{ cm}^{-1}$ region and 2.608×10^{19} molecules cm^{-2} and 1.60 p.p.m.v. from the $2,915.2$ – $2,922.0\text{ cm}^{-1}$ region. The average of these two determinations of the CH_4 tropospheric concentration in February 1981 is 1.58 p.p.m.v., slightly lower than published *in situ* measurements for the same time and at nearly the same latitude^{6–8}. However, within our estimated experimental uncertainty of 6% (discussed below), there is agreement in the results.

The results of the fits obtained for the Jungfraujoch and Kitt Peak spectra in the $2,903.0$ – $2,912.2\text{ cm}^{-1}$ region are shown in Figs 1 and 2. The measured intensities have been normalized to the highest value in the fitted region, and the residuals

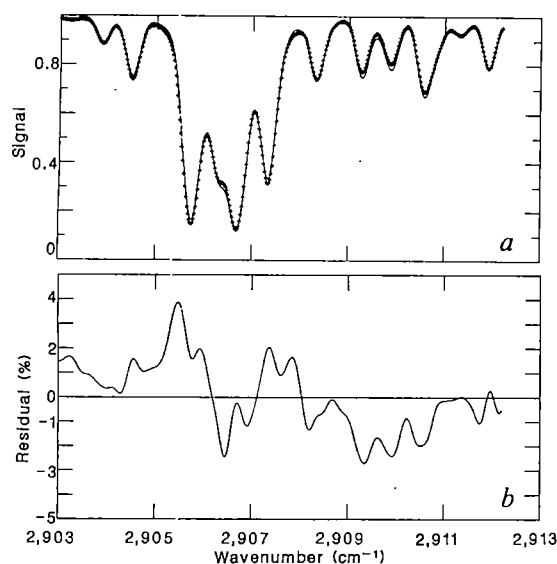


Fig. 2 Comparison (a) between the degraded Kitt Peak spectrum (solid line) and a least-squares best-fit to the data (plus symbols). Residuals (b) are observed-calculated values.

(observed minus calculated) are expressed as a percentage of the peak measured intensity. The standard deviations for the Jungfraujoch and Kitt Peak spectral fits are 1.19 and 1.44%, respectively. Although the agreement between the observed and best-fit calculated spectra is good for both data sets, some minor systematic discrepancies can be noted, especially for the higher air mass and higher signal-to-noise Kitt Peak data. The quality of the fitting results is about the same for the 2,915.2–2,922.0 cm^{-1} region.

Error analysis

A plot on an expanded scale of the observed and least-squares best-fit calculated spectra shows that a very good fit to the Jungfraujoch instrument function has been obtained with the gaussian line-shape model adopted in the analysis. However, it is important to test the sensitivity of the results to possible deviations from this model. Therefore, we repeated the Jungfraujoch retrieval for the lower wavenumber region assuming triangular and lorentzian line-shape functions. Again, a single adjustable width parameter was included in the analysis. Values for the CH_4 tropospheric mixing ratio and the standard deviation of the fit were, respectively, 1.15 p.p.m.v. and 1.26% for the triangular function and 1.24 p.p.m.v. and 1.70% for the Lorentz function, compared with 1.16 p.p.m.v. and 1.19% for the gaussian function. Plots of the results showed that the quality of the fits were nearly the same for the gaussian and triangular response functions, but that the strong wings modelled by the Lorentz function clearly did not represent satisfactorily the observational data. From these tests, we estimate that at most a 3% error in the retrieved mixing ratios can be caused by uncertainties in the line-shape function.

Scattered light and any nonlinearities in the detector-amplifier system are important sources of potential error. Both these effects are discussed in the solar atlas¹⁴, and produce negligible errors in the recording of the spectral data. The zero level was recorded at the beginning of the short section of spectrum analysed in this study. Therefore, the error in the zero level is also believed to be negligible. Although a number of solar lines can be identified in the 0.01- cm^{-1} resolution Kitt Peak spectra, their contribution to the total absorption in the analysed region is small and negligible error results from excluding them from our spectral simulation/retrieval algorithm.

In addition to the sensitivity studies already discussed, calculations similar to those presented in two previous studies^{34,36} were made to estimate the uncertainty in the CH_4 tropospheric mixing ratio caused by uncertainties in key parameters and by instrument noise. The parameters considered are the CH_4 line

Table 2 Systematic error sensitivity study

Error parameter	CH_4 tropospheric mixing-ratio error (%)	
	JF	KP
a, $\Delta S/S = +0.05$ for all CH_4 lines	-5	-5
b, $\Delta b_c/b_c = +0.05$ for all CH_4 lines	-2	-3
c, $\Delta n/n = +0.20$ for all CH_4 lines	-2	-2
d, $\Delta T = +2$ K for all layers	-1	-1
e, $\Delta P/P = +0.02$ (JF), $+0.01$ (KP) for all layers	-2	-1
f, 1.1% (1σ) gaussian instrument noise (JF only)	1	<1
g, Instrument line shape	3	<1
h, Vertical profile shape	<1	<1
i, r.s.s. of a-h	7	6

JF, Jungfraujoch; KP, Kitt Peak.

intensities S , the CH_4 air-broadened halfwidths at 296 K b_c (where b_c is the Lorentz half-linewidth at half absorption height per atmosphere), the temperature dependence of the air-broadened halfwidth n , the atmospheric temperature T and the atmospheric pressure P . The results of all the error studies are summarized in Table 2. For the Jungfraujoch data, the root of the sum of the squares (r.s.s.) of the individual uncertainty values is 7% or 0.08 p.p.m.v. The r.s.s. for the Kitt Peak spectrum is 6% or 0.09 p.p.m.v. These values have been adopted as the best estimate of the total experimental error. However, note that valid error estimates are difficult to determine for spectroscopic studies because of the difficulty in quantifying the errors for the many parameters involved in the analysis. The agreement between the Kitt Peak value and gas chromatograph determinations for the same epoch and nearly the same latitude⁶⁻⁸ suggests that we may have overestimated the total experimental error.

The calculations presented in Table 2 indicate that systematic errors in the line parameters produce about the same relative error in CH_4 concentration for both the Jungfraujoch and Kitt Peak spectra. Therefore, the average rate of increase in CH_4 has been determined more accurately than indicated by the total uncertainty limits assigned to both values. Assuming that errors in determining the rate of increase in CH_4 concentration result only from uncorrelated errors from sources c-h in Table 2, we calculate the average rate of increase of CH_4 concentration in the troposphere to be $1.1 \pm 0.2\%$ yr^{-1} from 1951 to 1981.

Comparison with previous analyses

It is important to note that the CH_4 column amount determined from our analysis is 71% of that inferred by Nielsen and Migeotte³⁷ from measurements of the $2\nu_3$ band in another set of 1951 Jungfraujoch solar spectra. In this earlier work, a curve of growth analysis method and the laboratory measurements of Goldberg *et al.*³⁸ were used to derive the total vertical column amount and the effective rotational temperature of CH_4 above the Jungfraujoch station. Although some of the discrepancy no doubt results from the use of the two different methods of analysis, we believe that most of the discrepancy is caused by the assumption of the Goldberg *et al.* intensities³⁸, which are considerably lower than several more recent studies.

Table 3 compares the Goldberg *et al.* intensities³⁸ with newer measurements³⁹⁻⁴² for the R0 and R5 lines used in Nielsen and

Table 3 Comparison of intensities reported for the R0 and R5 lines of the $2\nu_3$ band of $^{12}\text{CH}_4$

Ref.	Intensity ($\text{cm}^{-2} \text{atm}^{-1}$ at 296 K)	
	R0	R5
38	0.0098	0.0600
39	0.0095	
40	0.0134	0.0734
41	0.0166	0.0827
42	0.0128	

Migeotte's analysis³⁷. (The values have been converted to a standard set of intensity units to facilitate comparison.) The R10 line was also used by Nielsen and Migeotte³⁷ but, to our knowledge, there have been no more recent determinations of its intensity. Except for Fink *et al.*³⁹ results for R0, the newer intensity values are all considerably larger than those reported by Goldberg *et al.*³⁸, but there is considerable scatter in the measurements. A higher intensity for R0 of 0.0143 cm⁻² atm⁻¹ at 298 K has also been derived by J. S. Margolis (personal communication) from a preliminary analysis of new Kitt Peak laboratory spectra of the 2ν₃ band. As an additional check, we have analysed the R0 line in a Kitt Peak solar spectrum recorded with a zenith angle of 49.37° on 10 November 1981. To obtain a CH₄ tropospheric mixing ratio of 1.60 p.p.m.v., an intensity value of 0.0147 cm⁻² atm⁻¹ at 296 K is required, again considerably larger than the Goldberg *et al.*³⁸ intensity. If the Fink *et al.*³⁹ results are excluded, the average of the newer laboratory determinations is 1.40 times larger than the Goldberg *et al.*³⁸ values. Simply scaling the Nielsen and Migeotte³⁷ column amounts by the inverse of this factor produces a CH₄ vertical column amount of 1.49 × 10¹⁹ molecules cm⁻², corresponding to a CH₄ tropospheric mixing ratio of 1.14 p.p.m.v. The same intensities³⁸ were used by Goldberg⁴³ to analyse infrared solar spectra recorded from Lake Angelus, Michigan (elevation 296 m), and from Mt Wilson, California (elevation 1,750 m), in 1949–1950. If the same scaling factor for the intensity error is adopted along with the case B profile shape, we determine values of 2.28 × 10¹⁹ molecules cm⁻² and 1.17 p.p.m.v. and 1.85 × 10¹⁹ molecules cm⁻² and 1.15 p.p.m.v. for the CH₄ vertical column amount and tropospheric mixing ratio above Lake Angelus and Mt Wilson, respectively. These revised values for the ~1950 CH₄ tropospheric mixing ratio are in excellent agreement with the result of our detailed analysis of the Jungfraujoch spectra, but they should be regarded as subject to a large uncertainty because of the scatter in the intensity values reported for the 2ν₃ band. It is interesting to note that a gas chromatographic analysis of air in the troposphere in 1951 indicated a CH₄ mixing ratio of between 1.1 and 1.2 p.p.m.v.^{44,45}, in excellent agreement with our results.

Conclusions

The major finding reported in this paper, that historic solar spectra indicate a significantly lower concentration of CH₄ (1.14 p.p.m.v.) in 1951, has important implications for the fol-

lowing reasons: (1) the result extends the historic baseline of atmospheric measurements of CH₄; (2) the results reported here are consistent with a global increase of atmospheric CH₄ of 1.1 ± 0.2% yr⁻¹ from 1951 to 1981, assuming a constant rate of increase over this period. This suggests at least a 30-yr increase in atmospheric CH₄, a significantly longer time increase than previously reported, based on atmospheric measurements. It is interesting to note that the average CH₄ increase of 1.1 ± 0.2% yr⁻¹ from 1951 to 1981 reported here is comparable with the annual CH₄ increase deduced from several years of continuous gas chromatograph measurements in the late 1970s and early 1980s; (3) the earlier reported higher values of CH₄ deduced from ground-based infrared solar spectra^{37,43} can be significantly lowered using improved spectroscopic parameters; (4) the atmospheric lifetime of CH₄ is long (~7 yr) compared with the 1–2 yr required to mix a species uniformly throughout both hemispheres⁴⁶. Hence, a single measurement of CH₄, as reported here, is characteristic of its global concentration. Therefore, our measurement has important implications for the global budget of CH₄. The 30-yr increase of methane reported here may be caused by an increase in the global production rate of CH₄ (which includes anaerobic decomposition of organic matter, enteric fermentation in ruminants, biomass burning and natural gas leakage), a decrease in the global destruction rate of CH₄ (which is controlled by the reaction of CH₄ with the hydroxyl radical OH), or a combination of both^{47,48}. The implications of the Jungfraujoch spectra-deduced concentration of CH₄ and other trace radiative-active species and their impact on global species budgets and on the photochemistry of the atmosphere will be discussed in an accompanying paper⁴⁹.

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Free tropospheric carbon monoxide concentrations in 1950 and 1951 deduced from infrared total column amount measurements

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Published total vertical column amounts of carbon monoxide deduced from infrared solar spectra recorded during 1950–51 in the Swiss Alps have been reanalysed based on improved spectroscopic parameters for CO and a curve of growth. From a comparison of the 1950–51 results and modern measurements, an average increase of ~2% per year in the free tropospheric concentration of CO above Europe is estimated for 1950–77.

CARBON monoxide (CO) was discovered by Migeotte in the Earth's atmosphere by its absorption signature in infrared, ground-based spectra of the Sun¹. (Migeotte² also discovered methane (CH₄) using the same technique.) Carbon monoxide is an important species in the photochemistry of the troposphere. The reaction between CO and the hydroxyl radical (OH), the dominant oxidizing species in the troposphere, controls the global destruction of both of these species. The reaction between these two gases accounts for more than 80% of the global destruction of OH and the total destruction of CO, and the reaction between CO and OH which produces an atom of hydrogen (H) may also lead to the photochemical production of ozone (O₃) in the troposphere³.

Measurements indicate that at ground level, the mixing ratio of CO is between 120 and 200 parts per 10⁹ by volume (p.p.b.v.) in the Northern Hemisphere compared with 40–60 p.p.b.v. in the Southern Hemisphere³. The surface mixing ratio of CO is higher than in the free troposphere, at least for the Northern Hemisphere³. Measurements in the Northern Hemisphere indicate the background level of CO varies by about a factor of two with season^{4,5}, with a seasonal maximum in CO of about 150 p.p.b.v. in early spring and a seasonal minimum of about 70 p.p.b.v. in late summer at Cape Meares, Oregon, a remote site at 45° N (ref. 4).

There have been several recent studies on the possible temporal change of CO mixing ratios in the troposphere. Khalil and Rasmussen⁴ found a long-term increase of about 6% yr⁻¹ based on 44 months of continuous Cape Meares data (1979–82). Analyses of two other CO data sets by the same authors also indicate a long-term increase in tropospheric CO. The first of these two other CO data sets is unpublished and was obtained at Cape Grim, Tasmania (42° S) between October 1981 and April 1984. These results suggest that CO may be increasing by about 4% yr⁻¹ in the Southern Hemisphere. However, we note that Seiler *et al.*⁷ have compared Southern Hemispheric CO mixing ratios measured in 1971–72 with CO mixing ratios measured continuously at Cape Point (34° S) between 1978 and 1981 and conclude that Southern Hemisphere CO mixing ratios could not have changed by more than 5–10% over the past decade. Measurements of the total column amount of CO have been deduced from infrared spectroscopic measurements recorded between 1970 and 1982 (excluding a gap in the measurements between 1977 and 1980) in the Soviet Union^{8–11}. The reanalysis by Khalil and Rasmussen⁶ of the data reported by Dvoryashina *et al.*⁸ suggests that CO may have increased by 2% (±1%) yr⁻¹ since 1974, but there is no evidence for an increase in CO prior to 1974. The long-term increase of tropospheric CO suggested by some of these studies^{4,6} is very important and may indicate a significant anthropogenic perturbation to tropospheric composition and/or chemistry. Of equal importance is the conclusion that the long-term CO increase is not apparent in the measurement records in the Soviet Union prior to 1974.

As emphasized in these studies^{4,6}, it is very difficult to separate

the relatively small systematic long-term trend in background CO concentration from the large seasonal cycle and fluctuations in concentration caused by seasonal variations in CO production, destruction, and transport. For this reason, it is desirable to have continuous measurements from a remote site spanning several decades. Although no such database exists, we believe that important information on the long-term trend in CO concentration can be deduced from analysis of historic solar spectra. Since atmospheric concentrations of CO are difficult to store in containers¹², historic solar spectra may represent the only record of early CO concentrations. A number of infrared solar spectra obtained in the early 1950s show prominent telluric lines of CO and have been analysed for total CO column amounts^{13–16}. With the exception of the study by Benesch *et al.*¹³, these spectra were recorded near large urban areas, which are significant sources of CO.

The Benesch *et al.*¹³ measurements are unique in that they were obtained from spectra recorded at a remote, clean-air, high-altitude observatory in the Swiss Alps. We have previously analysed spectra obtained more than three decades ago at the same site to deduce the tropospheric CH₄ level¹⁷. In this article, we reanalyse the early CO column amount measurements of Benesch *et al.*¹³ using modern spectroscopic parameters and obtain estimates of the free tropospheric volume mixing ratio of CO above Europe on 9 days in 1950–51.

Measurements and analysis

The solar spectra analyzed by Benesch *et al.*¹³ were recorded at the Jungfraujoch Scientific Station in the Swiss Alps (46.5° N, 8.0° E, altitude 3,578 m) on three days in August 1950 and on six days between April and September 1951. The observations were recorded with a Pfund-type prism-grating spectrometer and with a Perkin-Elmer thermocouple as a detector. The instrumentation has been described in detail^{18,19} and a number of the spectra have been published in a solar atlas²⁰, including measurements which show prominent CO absorption lines on 25 April 1951 and 7 June 1951. Most of the spectra analyzed by Benesch *et al.*¹³ are unpublished.

Benesch *et al.*¹³ determined total vertical column amounts of CO (in units of cm-atm at STP) from measurements of the equivalent width of the R3 line of the (1-0) vibration-rotation band of ¹²C¹⁶O at 2,158.300 cm⁻¹. The column amounts were derived assuming that the absorption of the R3 line in the solar spectra occurs in the square root region of the curve of growth. In the square root region, the equivalent width W is given by^{15,21}

$$W = 2(Sb_L Lu)^{1/2} \quad (1)$$

where b_L is the Lorentz half-linewidth at absorption half height, S is the line intensity per molecule, L is Loschmidt's number, and u is the absorber column amount in units of cm-atm at STP. Equation (1) assumes the equivalent width has been obtained from an integration of the absorption over all frequen-

Table 1 Comparison of line parameters at 296 K for the R3 line of the (1-0) band of $^{12}\text{C}^{16}\text{O}$

Parameter	Units	Benesch <i>et al.</i> ¹³ Value	Present study	
			Value	Ref.
S	cm^{-1} molecule	2.91×10^{-19}	3.47×10^{-19}	Chackerian <i>et al.</i> ²²
b_L^0 (CO-air)	$\text{cm}^{-1} \text{ atm}^{-1}$	0.046	0.0672	Nakazawa & Tanaka ²⁴
n		0.0	0.75	Varanasi ²⁶

cies. The halfwidth b_L is related to the halfwidth per atmosphere b_L^0 at reference temperature T_0 by

$$b_L = b_L^0 \left(\frac{T}{T_0} \right)^{-n} p \quad (2)$$

where T is the temperature in K, p is the pressure in atm, and n is the coefficient of the temperature dependence of the halfwidth. Benesch *et al.*¹³ assumed the air-broadened halfwidth to be independent of temperature ($n=0$).

A recent comprehensive survey of the literature shows a large number of measurements of intensities and collisional broadening parameters for the 1-0 band of $^{12}\text{C}^{16}\text{O}$ (ref. 21). From these results, we have attempted to select the most accurate parameters for the R3 line. The adopted values converted to the standard reference temperature of 296 K are listed in Table 1 along with those assumed in the Benesch *et al.*¹³ analysis. The $^{12}\text{C}^{16}\text{O}$ line intensities of Chackerian *et al.*²² have a quoted absolute uncertainty of $\pm 3\%$. We multiplied the Chackerian *et al.*²² intensity for R3 by 0.98652 to account for the concentration of $^{12}\text{C}^{16}\text{O}$ in natural CO (ref. 23). The air-broadened halfwidth b_L^0 (CO-air) assumed in this study was calculated from the experimental halfwidths of Nakazawa and Tanaka²⁴ assuming the relation

$$b_L^0(\text{CO-air}) = 0.79 b_L^0(\text{CO-N}_2) + 0.21 b_L^0(\text{CO-O}_2) \quad (3)$$

where $b_L^0(\text{CO-N}_2)$ and $b_L^0(\text{CO-O}_2)$ are the experimental N_2 - and O_2 -broadened halfwidths, respectively. We note that the Varghese and Hanson²⁵ N_2 -broadened halfwidth for R3 agrees within 4% with the Nakazawa and Tanaka value²⁴ and that the calculated air-broadened halfwidth agrees closely with the value of $0.0695 \pm 0.0036 \text{ cm}^{-1} \text{ atm}^{-1}$ at 300 K determined for air-broadening by Varanasi²⁶.

Benesch *et al.*¹³ assumed an effective temperature of 230 K and an effective pressure of 0.3 atm for the CO molecules above the Jungfraujoch station. If these values are also adopted as a first approximation, the column amounts u_B derived by Benesch *et al.*¹³ can be compared to column amounts u_R determined with the revised spectroscopic parameters. Substituting the values from Table 1 into equations (1) and (2), we find for a given measured equivalent width that

$$\frac{u_R}{u_B} = 0.475 \quad (4)$$

Hence, by assuming values that are too low for both the R3 intensity and halfwidth, Benesch *et al.*¹³ overestimated the CO column amount above the Jungfraujoch station by about a factor of 2.

More accurate corrections to the Benesch *et al.*¹³ values have been obtained from a curve of growth derived from equivalent widths measured from synthetic spectra calculated with a 17-layer atmospheric model. Table 2 presents the pressure-temperature and CO reference mixing ratio profile assumed in the analysis. The pressures and temperatures are density-weighted values calculated from the 1976 US Standard Atmosphere²⁷. The reference CO mixing ratio profile adopted in the analysis is based on midlatitude Northern Hemisphere measurements. The stratospheric portion of this profile is taken from a set of reference gas concentration profiles²⁸ and is based on the measurements of Fabian *et al.*²⁹ and Ehhalt and Tönnissen³⁰. Below 11 km, a constant mixing ratio of 130 p.p.b.v. has been assumed based on an approximate average of free tropospheric measurements over Europe, although the actual measurements exhibit a wide scatter around the average value, as shown in

Fig. 10 of Logan *et al.*³. A Voigt profile has been assumed for the CO line shape.

Our spectral simulations indicate that the R3 CO line is slightly blended with a feature due to N_2O and solar CO on the low wavenumber wing and a feature due to N_2O on the high wavenumber wing. Also, the R3 line contains a contribution due to the absorption by the same transition in the solar atmosphere. Because the blends on both wings of the line were marked in the solar atlas²⁰ and identified in the list of measures³¹, we have assumed that Benesch *et al.*¹³ corrected their measured R3 line equivalent widths for both telluric and solar line absorption by these blending transitions. Therefore, only telluric absorption by the R3 line was included in our curve of growth calculations. The revised parameters in Table 1 were assumed along with the position and lower state energy from the 1982 Air Force Geophysics Laboratory line parameters compilation³².

The CO volume mixing ratio profile in Table 2 corresponds to a vertical column amount of 0.0512 cm-atm STP. To determine the curve of growth, a sequence of 10 synthetic spectra were calculated by scaling this mixing ratio distribution by multiplicative factors ranging from 0.1 to 2.4. Each spectrum has been convolved with a gaussian instrument function having a width corresponding to the resolution of the Jungfraujoch measurements as given in Table 1 of the solar atlas²⁰. Because of significant pressure broadening in the troposphere, absorption in the line wings is important in determining the total equivalent width. Since Benesch *et al.*¹³ did not discuss corrections to their equivalent widths for the contribution from the line wings, we assume their values were derived from integrating over only a finite wavenumber interval. From examination of the published solar spectra in this region^{13,20} and our calculated synthetic spectra, we estimated their integration limits to have been 2,157.5–2,159.1 cm^{-1} . Outside of this interval, absorption by the R3 line changes slowly with wavenumber and is overlapped by absorption from other lines in the Jungfraujoch solar spectra. We adopted these integration limits in analyzing the synthetic spectra and determined the background (measured 100% transmittance level) from a least-squares fit to data within 0.1 cm^{-1} of the integration limits. The derived equivalent widths are fairly insensitive to the adopted integration limits. For example, if the

Table 2 Atmospheric parameters assumed in the analysis

Layer boundaries (km)	Temperature (K)	Pressure (mbar)	CO mixing ratio (p.p.b.v.)
3.578–5.0	260.4	595.3	130
5.0–7.5	247.9	461.2	130
7.5–9.0	234.7	345.1	130
9.0–11.0	224.2	267.3	130
11.0–14.0	217.3	184.2	72.5
14.0–17.0	216.6	115.1	35.0
17.0–20.0	216.6	71.9	18.8
20.0–22.0	217.6	47.9	15.5
22.0–24.0	219.5	35.1	16.5
24.0–26.0	221.5	25.8	17.5
26.0–28.0	223.5	19.1	18.5
28.0–30.0	225.5	14.1	19.5
30.0–32.0	228.4	10.4	21.0
32.0–35.0	233.3	7.35	23.5
35.0–40.0	242.5	4.33	29.2
40.0–60.0	260.0	1.55	30.0
60.0–100.0	230.7	0.11	30.0

integration range is decreased to 2,157.6–2,159.0 cm^{-1} , the equivalent widths determined from the synthetic spectra decrease by 0.8–2.1%. An increase in the integration range to 2,157.4–2,159.2 cm^{-1} increases the calculated equivalent widths by 0.6–1.7%. As expected, the largest changes in equivalent width were found for the calculations with the largest CO profile multiplicative scaling factor.

For each of the 11 measurements reported by Benesch *et al.*¹³, a revised total vertical column amount and a corresponding free tropospheric volume mixing ratio of CO were derived from the curve of growth using the following steps:

- (1) The solar zenith angle was estimated from the date and approximate time of observation (listed in Fig. 5 of Benesch *et al.*¹³).
- (2) The CO column amount was calculated from the product of the secant of the solar zenith angle and the reported total vertical column amount.
- (3) From this result and the line parameters assumed by Benesch *et al.*¹³, the measured equivalent width was estimated using equation (1).
- (4) A revised CO column amount was derived from this equivalent width using the curve of growth.
- (5) The revised vertical column of CO was calculated by dividing the revised CO column amount by the secant of the solar zenith angle. The corresponding volume mixing ratio below 11 km is assumed to be the free tropospheric mixing ratio of CO for that date and time.

The revised total vertical column amounts and corresponding free tropospheric volume mixing ratios of CO are given in Table 3. For comparison, the total vertical column amounts of CO deduced by Benesch *et al.*¹³ are also listed. On the average, the revised values are 59% of the Benesch *et al.*¹³ column amounts. This result is slightly higher than the 47.5% estimate obtained from the simple scaling procedures discussed previously.

As a check on our curve of growth results, the region in the Jungfraujoch solar atlas²⁰ containing the R3 line of CO was analysed using the revised set of CO line parameters (Table 1) and the technique of nonlinear least squares spectral curve fitting. These spectral data were recorded at about 6:30 UT on April 25, 1951, corresponding to a solar zenith angle of 69.3° (measurement no. 5 in Table 3). Solar CO lines in the region were included in the fitting procedure as described by Rinsland *et al.*³³; otherwise, the analysis method was the same as that used to determine the 1951 CH_4 tropospheric mixing ratio from the Jungfraujoch solar spectra¹⁷. The fitting results are shown in Fig. 1. The free tropospheric mixing ratio of CO deduced from this spectral analysis is 99 p.p.b.v., somewhat lower than the value of 110 p.p.b.v. obtained from the curve of growth analysis for the same measurements.

It is also important to be sure that the mixing ratio results do not depend on the line selected for the analysis. Therefore, the spectral fitting procedure and a similarly revised set of CO line parameters have been applied to the analysis of the spectral

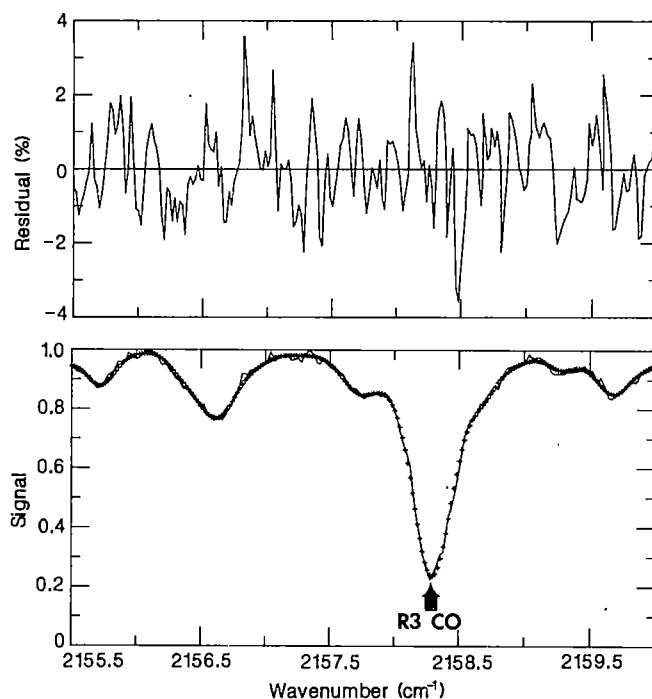


Fig. 1 Comparison between a solar absorption spectrum recorded at the Jungfraujoch Scientific Station on 25 April 1951 (solid line) and least-squares best-fit to the data (crosses). The measured spectrum was obtained about 6:30 UT at a solar zenith angle of 69.3° and has been normalized to the largest measured intensity in the fitted region. The residuals (observed minus calculated) are plotted at top.

interval containing the P14 line at 2,086.322 cm^{-1} and the P15 line at 2,082.002 cm^{-1} . These data were also recorded on 25 April 1951. The value of 103 p.p.b.v. determined from this analysis is in good agreement with the values obtained from the spectral fitting and curve of growth analyses of the R3 line (cited above).

The CO volume mixing ratios in Table 3 range from 30 to 110 p.p.b.v., a factor of 3.7 to 1, indicating large variability in CO abundance (the extreme limit of 4.4 to 1 deduced by Benesch *et al.*¹³ is reduced slightly by curve-of-growth effects). Although most of this variability is probably real, some of the scatter in the measurements is likely to be due to changes in the altitude of the tropopause (a constant value of 11 km has been assumed), errors in the equivalent width measurements of Benesch *et al.*¹³, and errors resulting from the assumption of a standard atmosphere pressure-temperature profile in the analysis. It is interesting to note that Benesch *et al.*¹³ observed significant changes in the CO total vertical amount on time scales of several hours. They were unable to find a meaningful correlation between these variations and meteorological data.

The CO mixing ratios observed on the 3 days of measurement in August 1950 are lower than those observed during August and September 1951 and much lower than all modern measurements of free tropospheric CO near the same latitude. Therefore, we investigated the available meteorological records for unusual conditions, for example very low tropopause altitude or intrusion of stratospheric air during a tropopause folding event. Surface weather maps for the 3 days of observation (J. Tierney, personal communication) show a weak anti-cyclonic circulation pattern near Switzerland with no evidence for the vigorous tropospheric depressions and associated fronts typically observed during a significant intrusion of stratospheric air into the troposphere³⁴. Radiosonde measurements from Payerne (46.5° N, 6.6° E) for 05 h U.T. indicate tropopause altitudes of 10.5 km for 19 August, 13.0 km for 21 August and 12.3 km for 30 August (no radiosonde measurements were made on 20 August, P. Hachler, personal communication), close to the nominal tropopause height of 11 km assumed in our calculations for all dates. However, the conventional temperature lapse rate

Table 3 Total vertical column amount u_{CO} (in cm-atm at STP) and corresponding free tropospheric mixing ratio β_{CO} of carbon monoxide above the Jungfraujoch Scientific Station in 1950–51

Measurement no.	Date	Time (UT)	Benesch <i>et al.</i> ¹³ u_{CO}	This study u_{CO}	β_{CO}
1	19 August 1950	12:00	0.029	0.018	46
2	20 August 1950	9:00	0.017	0.012	30
3	30 August 1950	8:30	0.020	0.013	32
4	24 April 1951	9:30	0.057	0.032	82
5	25 April 1951	6:30	0.075	0.043	110
6	7 June 1951	7:30	0.058	0.032	82
7	7 June 1951	13:00	0.068	0.038	98
8	14 August 1951	9:00	0.048	0.027	70
9	20 September 1951	8:30	0.068	0.038	96
10	20 September 1951	13:00	0.042	0.024	61
11	21 September 1951	16:00	0.043	0.024	62

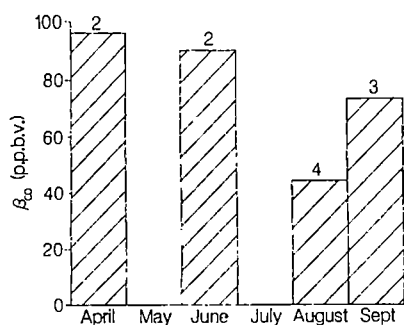


Fig. 2 Monthly averaged free tropospheric volume mixing ratios of CO deduced from the 1950–51 Jungfraujoch solar spectra. The number of measurements is shown for each month.

criterion used to define the tropopause altitude may be a poor indicator of the separation of stratospheric and tropospheric air during a major tropopause folding event³⁴. Therefore, potential temperature versus log. pressure plots were constructed, and these do not show the large low-altitude vertical gradients of isentropes characteristic of a tropopause fold³⁴. On the basis of the available evidence we have retained these three measurements in our analysis of the long-term trend, and we find it quite interesting that such very low CO mixing ratios apparently existed in the free troposphere above Europe in 1950.

In Fig. 2, all of the measurements within a given month have been averaged to evaluate the seasonal trend in CO. As can be seen, despite the limited number of measurements, there is some evidence for a seasonal cycle in the 1950–51 CO mixing ratios, with higher values in spring than in late summer and early fall, in rough agreement with modern studies^{4–6}. Unfortunately, the number of measurements and their distribution with season are insufficient and the scatter of the results is too large to define accurately the seasonal cycle of atmospheric CO in 1950 and 1951.

Fortuitously, the Jungfraujoch measurements are distributed at about the times of year when modern Northern Hemisphere measurements^{4,5} indicate the CO concentrations range from their highest values (early spring) to their lowest values (late summer). Therefore, a straight average of the 4-monthly means in Fig. 2 can be used to calculate a seasonally-averaged value with minimal sampling bias. The result is 76 p.p.b.v., a value considerably lower than most published measurements for the free troposphere over Europe (see Fig. 10 of Logan *et al.*³). The estimated uncertainty in the 1950–51 average is rather large (± 25 p.p.b.v.), primarily because of the large inferred variability, the limited number of measurements, uncertainties in the assumed CO vertical mixing ratio distribution (the measurements presented in Fig. 10 of Logan *et al.*³ and the measurements of Seiler and Fishman³⁵ show some evidence for a decrease of CO volume mixing ratio with altitude in the free troposphere), and uncertainties in the revised R3 CO spectroscopic line parameters. The assumptions adopted in the analysis to infer the Benesch *et al.*¹³ equivalent widths also contribute to the uncertainty.

Estimate of long-term (CO) trend

The most accurate estimate of the long-term trend in tropospheric CO concentration would result from comparison of the 1950–51 values with values deduced in the same way from modern Jungfraujoch solar spectra. This procedure would reduce potential systematic errors resulting from uncertainties in the assumed values for the spectroscopic line parameters and the CO vertical mixing ratio distribution and from potential systematic errors resulting from the method of analysis. Unfortunately, no modern Jungfraujoch spectra covering the CO region have been published. Therefore, we have been forced to assume modern CO measurement values from the literature.

Figure 10 of Logan *et al.*³ presents a number of free tropospheric measurements of CO obtained over Europe by Seiler *et al.*³⁶ in November and December 1976 (50–60° N) and by Fabian

*et al.*³⁷ in June and September 1977 (44° N). Near 6 km, the mean altitude of tropospheric CO molecules above the Jungfraujoch station (assuming the reference CO vertical distribution in Table 2), these measurements range from 75 to 175 p.p.b.v. with an approximate average value of 130 p.p.b.v. Measurements^{35,38,39} at other longitudes between 1971 and 1978 show similar average free tropospheric CO mixing ratios near 45° N (see Fig. 10 of Seiler and Fishman³⁵). Assuming an average of 130 p.p.b.v. for 1976–77 and our seasonally averaged mixing ratio of 76 p.p.b.v. for 1950–51, an average rate of CO increase of 2.1% yr⁻¹ is obtained for this 27-year period. Uncertainty in the average rate of increase results from uncertainties in the adopted average values for both 1950–51 and 1976–77. Assuming average values ranging from 115 to 145 p.p.b.v. for the latter period and average values for 1950–51 corresponding to the estimated uncertainty limits, the average rate of CO concentration increase for 1950–77 is calculated from the extremes of the uncertainty limits to be between 0.5–4.1% yr⁻¹.

Conclusions

The major conclusion of this study is that the seasonally averaged free tropospheric mixing ratio of CO above Europe in 1950–51 was 76 ± 25 p.p.b.v., a value considerably lower than most modern measurements for the same location. The deduced average rate of increase in free tropospheric CO above Europe is $\sim 2\%$ yr⁻¹ for 1950–77 and is consistent with more recent measurements of increasing CO concentration at sites in both the Northern and Southern Hemispheres^{4,6}. The primary strength of the present analysis is that the average rate of CO increase has been estimated from measurements separated by 27 years, over which time the small rate of increase in CO concentration has accumulated to a value which allows it to be separated fairly well from short-term fluctuations and the seasonal cycle of CO. Nonetheless, the accuracy in the estimated average rate of increase in free tropospheric CO over Europe is limited considerably by the large variability of CO, as deduced both from our analysis of the limited set of 11 measurements from 1950–51 and from more numerous recent measurements. Because of the large spatial as well as temporal variability of tropospheric CO, the globally averaged rate of change in CO concentration cannot be inferred from our results. However, when the published measurements of increasing CO at both Northern and Southern Hemisphere sites^{4,6} are considered in addition to our results, we believe there now exists considerable experimental evidence for a global increase in tropospheric CO concentration. Some implications of the Jungfraujoch spectra-deduced increases in tropospheric CO and CH₄ are discussed in the accompanying paper.⁴⁰

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The photochemistry of methane and carbon monoxide in the troposphere in 1950 and 1985

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The ~1%/year increase in tropospheric methane and ~2%/year increase in tropospheric carbon monoxide deduced from recent analyses of ground-based solar infrared spectra recorded in 1950 and 1951 have very important implications for the photochemistry and chemistry of the troposphere. Photochemical calculations indicate that as a result of the increase of methane and carbon monoxide since 1950-51, levels of the hydroxyl radical, the key species in the photochemistry of the troposphere, may have decreased by about 25%.

RECENT analyses of ground-based solar infrared absorption spectra recorded in 1950 and 1951 at the Jungfraujoch Scientific Station in the Swiss Alps (46.5°N, 3578 m altitude) have led to the discovery that 34-35 years ago, the mean tropospheric mixing ratio of methane (CH₄) was 1.14 ± 0.08 parts per million by volume (p.p.m.v.)¹ or only about 70% of its present day mixing ratio of 1.65 p.p.m.v. and that the average free tropospheric (~5 km) mixing ratio of carbon monoxide (CO) was only about half of its present day mixing ratio at that latitude². Extrapolating the 1951 data point to the present day value suggests that tropospheric CH₄ has increased at an average rate of about $1.1 \pm 0.2\%$ yr⁻¹ over the past 34 years. This rate of increase in CH₄ is comparable to the 1-2% per year increase in tropospheric CH₄ based on nearly continuous ground-based gas chromatograph measurements obtained since about 1977³⁻⁶, and is consistent with CH₄ levels measured in air bubbles embedded in polar ice cores^{7,8}. Extrapolating the 1950-51 data to present day values at the same latitude suggests that tropospheric CO has increased at an average rate of about 2% per year over the past 34-35 years, consistent with very recent continuous ground-based measurements of CO obtained over a $3\frac{1}{2}$ year period (1979-82)⁹.

In this article, we consider the implications of the 1950 and 1951 measurements on the ground production rates of CH₄ and CO and on the tropospheric photochemical coupling between these two gases. At the outset, it must be emphasized that the troposphere is a strongly coupled chemical system of several dozen interacting species controlled by many different processes, including natural and anthropogenic emissions, photochemical and chemical processes, and various heterogeneous loss processes^{10,11}. There are many deficiencies and uncertainties in our understanding of the composition and chemistry of the present-day troposphere. Trying to understand the composition and chemistry of the troposphere more than three decades ago based on measurements of only two species is very difficult at the least. We know nothing about the concentrations of the other several dozen tropospheric species. While our analysis must be considered somewhat speculative, we believe that the analysis and interpretation of historic solar spectra using photochemical

models offer a new and powerful technique to study changes in tropospheric composition and chemistry over a period of several decades.

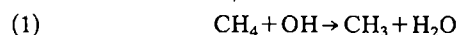
Photochemical calculations presented here were obtained with a one-dimensional photochemical model of the troposphere¹²⁻¹⁵. The version of the model used in these calculations contains 80 species from the following species families: hydrogen, nitrogen, oxygen, carbon, ammonia, sulphur and halogen (chlorine, bromine and iodine). The non-methane hydrocarbon chemistry^{13,14} was not included in the present calculations to reduce computational costs. The photochemical production of CO resulting from the oxidation of natural and anthropogenic nonmethane hydrocarbons is included in the surface flux boundary condition for CO, as will be described. The calculation of photolysis rates includes the effect of molecular absorption, Rayleigh scattering, Mie scattering, and surface albedo, based on a detailed radiative transfer model¹⁶ which has been incorporated into our photochemical model. The radiative transfer calculation uses optical depths for ozone absorption, Rayleigh scattering, and Mie scattering¹⁷. A major change in the present version of the model is the inclusion of CH₄ as a chemically active species whose vertical profile is calculated using a continuity-transport equation with a surface flux of CH₄ as the lower boundary condition. In earlier versions of the model, the vertical distribution of CH₄, a long-lived gas, was specified as an input parameter, as it is in almost every tropospheric photochemical model. For present day conditions, the following surface boundary conditions were specified for 45°N latitude: CH₄, upward flux of 1.5×10^{11} molecules cm⁻² s⁻¹ (which results in a surface mixing ratio of 1.65 p.p.m.v., the present-day value); CO, upward flux of 6.0×10^{11} molecules cm⁻² s⁻¹ (see below); NO_x (NO + NO₂), upward flux 5.0×10^9 molecules cm⁻² s⁻¹; HNO₃, downward flux of 1.55×10^9 molecules cm⁻² s⁻¹; and O₃, fixed-number density of 7.5×10^{11} molecules cm⁻³. At the tropopause (10 km), the following non-zero boundary conditions were imposed: NO_x, downward flux across tropopause of 2.0×10^7 molecules cm⁻² s⁻¹; HNO₃, downward flux across tropopause of 1.2×10^8 molecules cm⁻² s⁻¹; and O₃, fixed number density of 1.0×10^{12} molecules cm⁻³. The boundary condi-

tions for NO_x , HNO_3 and O_3 are typical values used in most photochemical models¹⁰.

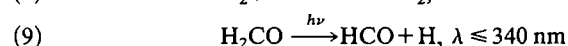
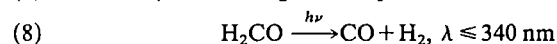
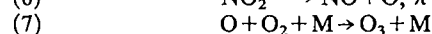
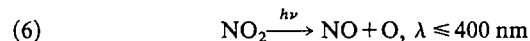
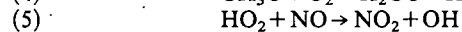
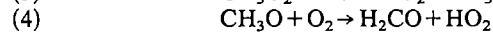
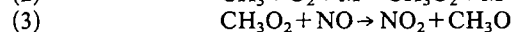
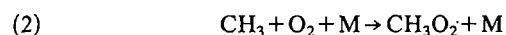
Methane is produced at the Earth's surface primarily by biological fermentation in anaerobic environments such as wetlands and rice fields; in ruminating animals, mostly cattle; and by biomass burning and natural gas leakage¹⁸. Methane is not produced photochemically or chemically in our strongly oxidizing atmosphere. Carbon monoxide is produced photochemically in the atmosphere via the methane oxidation chain¹⁹⁻²³. The oxidation of methane may be the largest source of CO, and may account for as much as 400–1,000 megatonnes of CO yr^{-1} (ref. 10). CO also results from other natural and anthropogenic activities. The estimated range for other known sources of CO (in units of $\text{Mt}(\text{CO}) \text{ yr}^{-1}$) are: oxidation of natural nonmethane hydrocarbons = 280–1,200; fossil fuel use = 400–1,000; forest clearing = 200–800; savanna burning = 100–400; emissions by plants = 50–200; oxidation of anthropogenic nonmethane hydrocarbons = 0–180; wood used as fuel = 25–150; ocean = 20–80; and forest wildfires = 10–50 (ref. 10). The range for the sum of the above CO sources other than the methane oxidation chain component, is 1,085–4,060 $\text{Mt}(\text{CO}) \text{ yr}^{-1}$ (equivalent to about $2\text{--}6 \times 10^{11} \text{ CO molecules cm}^{-2} \text{ s}^{-1}$). In a recent photochemical study, surface CO fluxes ranging between 1 and $10 \times 10^{11} \text{ CO molecules cm}^{-2} \text{ s}^{-1}$ were used²⁴. In our photochemical calculations for the present-day troposphere, we have assumed a surface CO flux which appears appropriate for 45°N , of $6 \times 10^{11} \text{ CO molecules cm}^{-2} \text{ s}^{-1}$. The Jungfraujoch spectra were obtained at a latitude of 46.5°N , just about the latitude of maximum CO concentration in the troposphere¹⁰. The CO produced via the methane oxidation chain is calculated explicitly as a photochemical production component in the model and is not included in the CO surface flux.

The apparent increase in tropospheric levels of CH_4 and CO over the past 35 years may have resulted from the following factors: (1) an increase in the global production rate of either, or both CH_4 and CO; (2) a decrease in the global destruction rate of either, or both CH_4 and CO; or (3) a combination of both (1) and (2), since both CH_4 and CO are closely coupled photochemically via the methane oxidation chain and through the hydroxyl radical (OH), the dominant destruction mechanism for both CH_4 and CO in the troposphere.

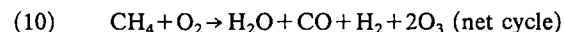
As mentioned above, the methane oxidation chain, which is initiated by the reaction of CH_4 with OH, and leads to the production of CO via the photolysis of formaldehyde (H_2CO), photochemically couples CH_4 and CO¹⁸⁻²³.



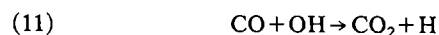
The reaction of CH_4 with OH is the dominant destruction mechanism for CH_4 in the troposphere, and is also a significant loss mechanism for tropospheric OH. The methyl radical (CH_3) formed in reaction (1) continues the methane oxidation chain, which is illustrated in the following reactions:



The methane oxidation chain leads to the photochemical production of CO, H_2 and O_3 , and may be represented by the following net cycle:



Just as the reaction with OH (reaction 1) is the dominant destruction mechanism for CH_4 , the destruction of CO is also controlled by its reaction with OH:



The reactions of OH with CH_4 (reaction 1) and CO (reaction 11) control the global destruction of OH, the dominant oxidizing species in the troposphere, and, hence, the dominant tropospheric scavenging species. The reactions of OH with CH_4 and CO account for about 97% of the total tropospheric destruction of OH (for noontime, groundlevel conditions at 45°N , CO accounts for 81% and CH_4 16% of the total destruction of OH)¹⁰. The reaction between CH_4 and OH is such a dominant loss mechanism for CH_4 that more than 90% of the global destruction of CH_4 occurs in the troposphere, with nearly 80% occurring in the lowest atmospheric scale height¹⁸. Several model studies have considered the photochemical coupling of CH_4 , CO, and OH and the effects of anthropogenic emissions of CO on the CH_4 —CO—OH chemical system²⁴⁻²⁶.

Photochemical calculations can determine the surface flux of CH_4 (or CO) required to maintain a particular tropospheric mixing ratio of CH_4 (or CO). The critical parameter in these calculations is the vertical distribution of OH throughout the troposphere. Our photochemical calculations indicate that at 45°N (neglecting advection), the measured mixing ratio of CH_4 (1.65 p.p.m.v.) is maintained by a surface flux of $1.5 \times 10^{11} \text{ molecules cm}^{-2} \text{ s}^{-1}$ ($645 \text{ Mt}(\text{CH}_4) \text{ yr}^{-1}$) and that a CO surface flux of $6 \times 10^{11} \text{ molecules cm}^{-2} \text{ s}^{-1}$ ($4,200 \text{ Mt}(\text{CO}) \text{ yr}^{-1}$) results in a surface mixing ratio of about 180 parts per 10^9 by volume (p.p.b.v.) decreasing to about 130 p.p.b.v. at 5 km. For this calculation, the OH concentration was found to decrease from $2.9 \times 10^6 \text{ cm}^{-3}$ at the surface to $2.0 \times 10^6 \text{ cm}^{-3}$ at 1 km and then range between 1.0 and $1.5 \times 10^6 \text{ cm}^{-3}$ between 2 and 10 km. The total OH column in the troposphere was $1.6 \times 10^{12} \text{ cm}^{-2}$. The calculation of the OH profile in the troposphere depends on several parameters, including the level of incident solar radiation, which photodissociates tropospheric ozone producing excited oxygen atoms, which then react with water vapor producing OH. The level of incident radiation available for the photodissociation of ozone is a function of season (solar declination), latitude, time of day, and the scattering properties of the atmosphere, including the surface albedo¹². Time-dependent calculations indicate that at the surface at 45°N , the noontime OH concentrations are about $2 \times 10^6 \text{ cm}^{-3}$ for a surface albedo of 10%¹⁰. Photochemical calculations indicate that the concentration of OH increases as the surface albedo is increased¹². For the calculations in this work we have assumed summer conditions (solar declination of 22.5°N) at 45°N with a surface albedo of 25%. These parameters tend to maximize the calculated values of OH. Our calculated OH values are consistent with time-dependent calculations¹⁰ and with calculations with a varying surface albedo¹².

We also investigated the sensitivity of the CH_4 —CO—OH system to changes in the prescribed surface fluxes of CO and CH_4 . We varied the surface CO flux from 4 to $8 \times 10^{11} \text{ molecules cm}^{-2} \text{ s}^{-1}$ (at 45°N , the CO mixing ratio is maintained by a surface CO flux of $6 \times 10^{11} \text{ molecules cm}^{-2} \text{ s}^{-1}$ and a surface CH_4 flux of $1.5 \times 10^{11} \text{ molecules cm}^{-2} \text{ s}^{-1}$). In all of these calculations, we used a surface CH_4 flux of $1.5 \times 10^{11} \text{ molecules cm}^{-2} \text{ s}^{-1}$. The surface concentrations of CO, CH_4 , and OH as a function of the surface CO flux are summarized in Table 1. These calculations

Table 1 Sensitivity of CO, CH_4 and OH to the CO surface flux

CO surface flux (molecules $\text{cm}^{-2} \text{ s}^{-1}$)	Surface mixing ratio		Surface density OH (cm^{-3})
	CO (p.p.b.v.)	CH_4 (p.p.b.v.)	
4×10^{11}	107.0	1.321	3.46×10^6
5×10^{11}	141.1	1.479	3.14×10^6
6×10^{11} *	180.9	1.654	2.85×10^6
7×10^{11}	226.3	1.840	2.60×10^6
8×10^{11}	276.6	2.027	2.38×10^6

For all of these calculations, a CH_4 surface flux of $1.5 \times 10^{11} \text{ molecules cm}^{-2} \text{ s}^{-1}$ was used.

* Standard present-day run.

Table 2 Sensitivity of CH₄, CO and OH to the CH₄ surface flux

CH ₄ surface flux (molecules cm ⁻² s ⁻¹)	Surface mixing ratio		
	CH ₄ (p.p.m.v.)	CO (p.p.b.v.)	Surface density OH (cm ⁻³)
1.0 × 10 ¹¹	0.984	155.0	3.14 × 10 ⁶
1.5 × 10 ¹¹ *	1.654	180.9	2.85 × 10 ⁶
2.0 × 10 ¹¹	2.398	205.6	2.64 × 10 ⁶

For all of these calculations, a CO surface flux of 6×10^{11} molecules cm⁻² s⁻¹ was used.

* Standard present-day run.

tions indicate that if the CO surface flux is doubled (from 4 to 8×10^{11} molecules cm⁻² s⁻¹), the surface CO mixing ratio increased by a factor of 2.6, the surface CH₄ mixing ratio increased by a factor of 1.5 and the OH number density decreased to 69% of its original value. For comparison, in an earlier study²⁴, it was found that by doubling the CO surface flux (from 5 to 10×10^{11} molecules cm⁻² s⁻¹), the surface CO mixing ratio increased by a factor of 2.7, the surface CH₄ mixing ratio increased by a factor of 1.6, and the surface OH number density decreased to 62% of its original value. Similar results were also found in this study for a doubling of the CO surface flux from 1 to 2×10^{10} molecules cm⁻² s⁻¹ (ref. 24).

We also varied the surface CH₄ flux from 1.0 to 2.0×10^{11} molecules cm⁻² s⁻¹ while holding the surface CO flux constant at 6×10^{11} molecules cm⁻² s⁻¹. The surface concentrations of CH₄, CO, and OH as a function of the surface CH₄ flux are summarized in Table 2. These calculations indicate that a doubling of the surface CH₄ (from 1 to 2×10^{11} molecules cm⁻² s⁻¹) results in an increase in the surface CH₄ mixing ratio of a factor of 2.4, an increase in the surface CO mixing ratio of a factor of 1.3, and a decrease in the surface OH number density to 84% of its original value.

Decreased levels of CH₄ and CO in the 1950–51 troposphere may be attributable to a decreased surface flux of CH₄ and/or CO, due to the chemical coupling via the methane oxidation chain and OH destruction. In our calculations, we have assumed that surface fluxes of both CH₄ and CO have increased as a result of anthropogenic perturbations to the production of both gases. Calculations were performed to determine the fluxes of both CH₄ and CO consistent with the lowered CH₄ and CO mixing ratios deduced from the 1950 and 1951 groundbased solar infrared spectra, that is, CH₄ = 1.14 p.p.m.v. and CO about 65 p.p.b.v. in the free troposphere (5 km), which is within the uncertainty in the spectral determination (76 ± 25 p.p.b.v.)². Our results indicate that a CH₄ mixing ratio of 1.14 p.p.m.v. is maintained by a flux of 1.35×10^{11} cm⁻² s⁻¹ ($580 \text{ Mt}(\text{CH}_4) \text{ yr}^{-1}$) and the CO mixing ratio is maintained by a flux of 4.0×10^{11} cm⁻² s⁻¹ ($2,800 \text{ Mt}(\text{CO}) \text{ yr}^{-1}$). These calculations indicate that while the CH₄ mixing ratio increased about 44% from 1951 to the present (1.14 p.p.m.v. to 1.65 p.p.m.v.), the 45° N CH₄ flux increased by only 11% (1.35 to 1.50×10^{11} molecules cm⁻² s⁻¹). During this same period, the CO free tropospheric (5 km) mixing ratio increased by about 100% (from about 65 to about 130 p.p.b.v.), while the 45° N CO flux only increased by 50% (4.0 to 6.0×10^{11} molecules cm⁻² s⁻¹). For this calculation, the OH concentration was found to decrease from 3.6×10^6 cm⁻³ at the surface to 2.6×10^6 cm⁻³ at 1 km and then range between 1.3 and 1.9×10^6 cm⁻³ between 2 and 10 km. The total OH column in the troposphere was 2.1×10^{12} cm⁻³.

Photochemical calculations indicate at 45° N, the OH column in the troposphere decreased by about 25% (from 2.1×10^{12} to 1.6×10^{12} cm⁻²) from 1950 to the present, as a result of increased levels of CH₄ and CO in the troposphere. It is interesting to note that in a recent paper, a decrease in tropospheric OH of between 20 and 30% over the last 130 years was estimated as a result of increasing levels of CH₄²⁷. What is particularly interesting is that this estimate was based on two entirely different methods of analysis (on a mass balance of CH₄ in the

Table 3 Sensitivity of calculated CH₄, CO and OH to reduced NO_x and O₃ in 1950–51 troposphere

O ₃ Reduction	Reduced O ₃ (fixed NO _x)		Surface OH
	Surface CH ₄	CO at 5 km	
–10%	+6%	+8%	–8%
–25%	+17%	+20%	–19%
–50%	+40%	+47%	–38%

NO _x Reduction	Reduced NO _x (fixed O ₃)		Surface OH
	Surface CH ₄	CO at 5 km	
–10%	+2%	+2%	–1%
–25%	+5%	+5%	–6%
–50%	+13%	+13%	–17%

Values are calculated percentage change from standard 1950–51 calculation. In a standard run, the surface O₃ mixing ratio is 29.5 p.p.b.v. and the surface NO_x mixing ratio is 0.5 p.p.b.v.

past and present atmosphere and on calculations of the lifetimes of CH₄ in the past and present atmosphere), neither of which involve photochemical calculations, as does the present analysis. Hence, increasing levels of CH₄ and CO clearly impacted the scavenging ability of OH, the major chemical scavenger in the troposphere.

The calculated concentrations of CH₄, CO and OH are sensitive to the tropospheric levels of the nitrogen oxides, NO_x (NO + NO₂) and ozone (O₃)^{10,11}. Unfortunately, the 1950–51 spectra used to deduce levels of CH₄ and CO can provide no information on tropospheric NO_x and O₃. In fact, there does not appear to be any data base to provide this information. However, we can perform photochemical calculations to determine the sensitivity of the calculated concentrations of CH₄, CO and OH to changes in NO_x and O₃. It appears that both NO_x and O₃ in the troposphere are impacted by anthropogenic activities^{10,11}, and, hence we would expect lower concentrations in the 1950–51 time period. Therefore, we reduced present-day levels of NO_x by 10%, 25% and 50%, while keeping O₃ at the present-day level and then reduced present-day levels of O₃ by 10%, 25% and 50%, while keeping NO_x at the present-day level. The sensitivity of calculated levels of CH₄ and CO on other tropospheric species is summarized in reactions (1) to (11). The concentration of tropospheric OH is given by the following expression, with the OH production terms in the numerator, and the OH destruction terms in the denominator¹⁰:

$$(12) [\text{OH}] =$$

$$\frac{2k[\text{O}(^1\text{D})][\text{H}_2\text{O}] + [\text{HO}_2]\{k[\text{O}_3] + k[\text{NO}]\} + 2J[\text{H}_2\text{O}_2]}{k[\text{CO}] + k[\text{CH}_4]}$$

In this equation, k is the kinetic chemical reaction rate and J is the photolysis rate of H₂O₂. In the lower tropical troposphere, the production of OH occurs mainly by the first term in the numerator, with smaller contributions from the other three terms. Contributions from O₃ and NO grow in importance with latitude reflecting an increase in O₃ and NO due to anthropogenic activities. The photolysis of O₃ leads to the production of excited oxygen atoms (O¹D), which react with water vapour forming OH. Hence, OH production increases with increasing levels of O¹D and H₂O, as indicated by the first OH production term in the numerator. The concentration of OH is independent of NO for NO levels below about 10 parts per 10¹² by volume (p.p.t.v.); OH increases as a function of NO between 10 and 500 p.p.t.v.; and decreases as NO increases above about 1 p.p.b.v.¹⁰ The production of OH via the photolysis of hydrogen peroxide (H₂O₂), represented by the fourth term in the numerator, is a significant source of OH in the upper troposphere¹⁰. Also note that the loss of OH is controlled by its reactions with CH₄ and CO, as shown in the denominator. The results of these sensitivity calculations for reduced tropospheric levels of O₃ and NO_x are summarized in Table 3. Inspection of this table indicates that surface OH concentrations decrease with reduced levels of O₃ and NO_x, as predicted by equation

(12). For all of the reduced levels of O_3 and NO_x , except for the 50% O_3 reduction case, the decrease in OH is less than 20% of the standard 1950 calculation, indicating that OH levels may have indeed decreased from 1950 to the present day, as CH_4 and CO levels increased. The variation of OH as a function of NO_x level summarized in Table 3 is in good agreement with earlier sensitivity calculations^{10,24}.

Photochemical calculations of present-day levels of CH_4 and CO compared to levels of these gases deduced from 1950 and 1951 ground-based solar infrared spectra^{1,2} suggest that at 45° N: (1) the lower surface flux of CH_4 may have increased by about 11% from 1951 to the present, although the CH_4 mixing ratio increased by about 44%; (2) the lower surface flux of CO may

have increased by about 50% from 1950–51 to the present, although the free tropospheric (~5 km) CO mixing ratio increased by about 100%; and (3) increased tropospheric levels of CH_4 and CO from 1950–51 to the present resulted in about a 25% decrease in the tropospheric column of OH, and, hence, impacted the ability of the major tropospheric chemical scavenger to scavenge. Finally, the analysis presented here suggests that historic spectra coupled with photochemical calculations may provide important and previously unattainable information on global production of atmospheric gases over time periods of decades. This information may permit an assessment of the role of anthropogenic emissions to the global budgets of atmospheric species over these time periods.

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Transposition of structural genes to an expression sequence on a linear plasmid causes antigenic variation in the bacterium *Borrelia hermsii*

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In Borrelia hermsii, a spirochaete that causes relapsing fever, the switch between expression of two frequent variable major protein (VMP) types (7 and 21) is associated with a DNA rearrangement. Both cell types 7 and 21 contain untranscribed 7 and 21 VMP genes on linear plasmids. The serotype 7 cells contain an additional copy of the 7 VMP gene fused to an expression sequence on another linear plasmid. Switching to the 21 serotype involves removal of the transcribed 7 VMP gene and fusion of a copy of the 21 VMP gene to this same expression sequence. Thus recombination between linear plasmids can activate different VMP genes.

PATHOGENIC microorganisms have strategies to escape the host's antibody response. The selective pressure to develop evasive mechanisms is especially strong for extracellular pathogens that cycle between the vertebrate bloodstream and haematophagous arthropods: the longer the parasite survives in the bloodstream, the greater the probability of transmission through an arthropod to another animal. One of the strategies that these pathogens have developed is antigenic variation: a small fraction of multiplying cells undergoes a genetic change leading to the synthesis of a new and antigenically distinct surface protein. Those cells possessing novel serotypic determinants escape the immune response that is directed against the infecting serotype. The consequence to the host of antigenic variation is a relapsing or chronic illness.

Multiphasic antigenic variation has been described in eukaryotic and prokaryotic organisms. Salivarian trypanosomes such

as *Trypanosoma brucei* have a large assortment of surface glycoprotein genes that can be expressed in the host¹. Surface proteins of the bacterium *Neisseria gonorrhoeae* can vary during infection of human mucosa². Another group of bacteria, the borreliae, change their surface antigens during the course of relapsing fever and, like trypanosomes, are blood-borne and transmitted by vector³.

We are studying the molecular basis for antigenic variation in a *Borrelia* sp. for several reasons. (1) The antigenic repertoire is elaborate: 26 variant serotypes have derived from one serotype^{4,5}. (2) Infection in mice can be started with a single *Borrelia*⁴. (3) The organism can be cultivated *in vitro*⁶. (4) The variable antigens are abundant surface proteins that differ extensively in their primary structures^{7–9}. (5) Antigenic variation has been observed *in vivo* and *in vitro* at a frequency of 10^{-4} (ref. 4). These properties allow the isolation of populations of clonal origin, development of specific antibody and nucleic acid probes, and analysis of genetic material before and after the antigenic switch.

Recently, it was demonstrated that switching between three

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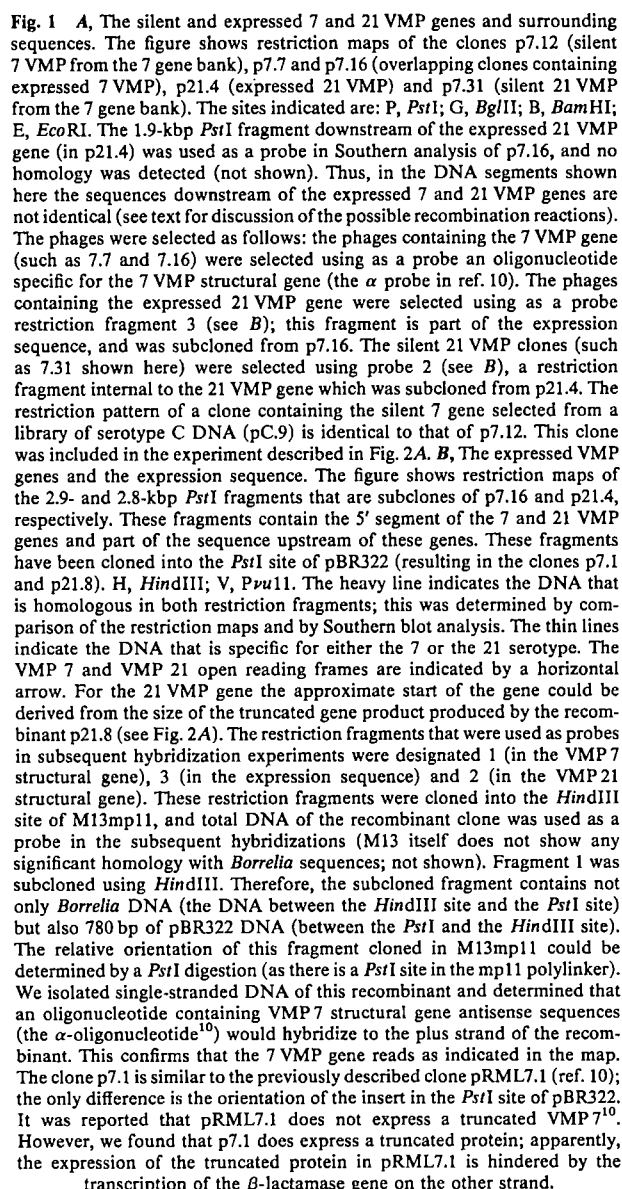
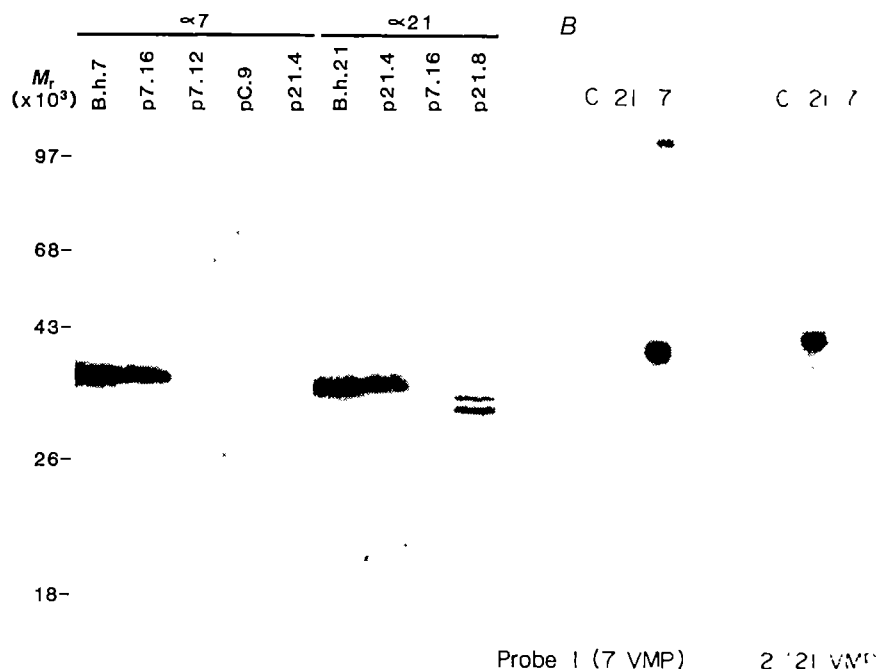


Fig. 1 A. The silent and expressed 7 and 21 VMP genes and surrounding sequences. The figure shows restriction maps of the clones p7.12 (silent 7 VMP from the 7 gene bank), p7.7 and p7.16 (overlapping clones containing expressed 7 VMP), p21.4 (expressed 21 VMP) and p7.31 (silent 21 VMP from the 7 gene bank). The sites indicated are: P, *Pst*I; G, *Bgl*II; B, *Bam*HI; E, *Eco*RI. The 1.9-kbp *Pst*I fragment downstream of the expressed 21 VMP gene (in p21.4) was used as a probe in Southern analysis of p7.16, and no homology was detected (not shown). Thus, in the DNA segments shown here the sequences downstream of the expressed 7 and 21 VMP genes are not identical (see text for discussion of the possible recombination reactions). The phages were selected as follows: the phages containing the 7 VMP gene (such as 7.7 and 7.16) were selected using as a probe an oligonucleotide specific for the 7 VMP structural gene (the α probe in ref. 10). The phages containing the expressed 21 VMP gene were selected using as a probe restriction fragment 3 (see B); this fragment is part of the expression sequence, and was subcloned from p7.16. The silent 21 VMP clones (such as 7.31 shown here) were selected using probe 2 (see B), a restriction fragment internal to the 21 VMP gene which was subcloned from p21.4. The restriction pattern of a clone containing the silent 7 gene selected from a library of serotype C DNA (pC.9) is identical to that of p7.12. This clone was included in the experiment described in Fig. 2A. B, The expressed VMP genes and the expression sequence. The figure shows restriction maps of the 2.9- and 2.8-kbp *Pst*I fragments that are subclones of p7.16 and p21.4, respectively. These fragments contain the 5' segment of the 7 and 21 VMP genes and part of the sequence upstream of these genes. These fragments have been cloned into the *Pst*I site of pBR322 (resulting in the clones p7.1 and p21.8). H, *Hind*III; V, *Pvu*II. The heavy line indicates the DNA that is homologous in both restriction fragments; this was determined by comparison of the restriction maps and by Southern blot analysis. The thin lines indicate the DNA that is specific for either the 7 or the 21 serotype. The VMP 7 and VMP 21 open reading frames are indicated by a horizontal arrow. For the 21 VMP gene the approximate start of the gene could be derived from the size of the truncated gene product produced by the recombinant p21.8 (see Fig. 2A). The restriction fragments that were used as probes in subsequent hybridization experiments were designated 1 (in the VMP 7 structural gene), 3 (in the expression sequence) and 2 (in the VMP 21 structural gene). These restriction fragments were cloned into the *Hind*III site of M13mp11, and total DNA of the recombinant clone was used as a probe in the subsequent hybridizations (M13 itself does not show any significant homology with *Borrelia* sequences; not shown). Fragment 1 was subcloned using *Hind*III. Therefore, the subcloned fragment contains not only *Borrelia* DNA (the DNA between the *Hind*III site and the *Pst*I site) but also 780 bp of pBR322 DNA (between the *Pst*I and the *Hind*III site). The relative orientation of this fragment cloned in M13mp11 could be determined by a *Pst*I digestion (as there is a *Pst*I site in the mp11 polylinker). We isolated single-stranded DNA of this recombinant and determined that an oligonucleotide containing VMP 7 structural gene antisense sequences (the α -oligonucleotide¹⁰) would hybridize to the plus strand of the recombinant. This confirms that the 7 VMP gene reads as indicated in the map. The clone p7.1 is similar to the previously described clone pRML7.1 (ref. 10); the only difference is the orientation of the insert in the *Pst*I site of pBR322. It was reported that pRML7.1 does not express a truncated VMP7¹⁰. However, we found that p7.1 does express a truncated protein; apparently, the expression of the truncated protein in pRML7.1 is hindered by the transcription of the β -lactamase gene on the other strand.

Similar conclusions were drawn from Western blot analysis of *E. coli* strains containing p7.1 or p21.8. Figure 2A shows that

Fig. 2 A, Western blot analysis of lysates of *B. hermsii* or of *E. coli* containing recombinant plasmids, with *Borrelia* DNA. Monoclonal antibody H12436 (9) is anti-VMP 7 ($\alpha 7$; left-hand panel); monoclonal antibody H10022 (9) is anti-VMP 21 ($\alpha 21$; right-hand panel). Lanes contained lysates of *B. hermsii* serotypes 7 (B.h.7) or 21 (B.h.21) or lysates of *E. coli* JM101 containing p7.16, p7.12, pC.9, p21.4 or p21.8. On the left are shown the relative migrations of pre-stained M_r standards (6041SA; BRL). *B. hermsii* cells were cultured, collected and lysed as described previously^{9,10}. The *E. coli* were grown in CYHM medium (1% Difco casamino acid, 0.5% Difco Bacto yeast extract, 50 mM NaCl, 25 mM KCl, 10 mM MgCl₂ and 20 mM HEPES pH 7.4). Harvested cells were resuspended in phosphate-buffered saline containing 5 mM MgCl₂ and 20 $\mu\text{g ml}^{-1}$ of phenylmethylsulphonyl fluoride and incubated for 15 min at 25 °C, then centrifuged. The pellet was resuspended in sample buffer containing 1% SDS and boiled for 5 min. The lysate was cleared by centrifugation; lysate components were analysed by Western blot analysis as described previously⁹. The blocking and antibody incubation solution comprised 2% bovine serum albumin in 50 mM Tris-HCl pH 7.6, 150 mM NaCl, 5 mM EDTA (TSE); the washing and ¹²⁵I-protein A incubation solution comprised 0.05% Tween 20 in TSE. **B**, Northern blot analysis of RNA from the three serotypes 7, 21 and C. The left-hand panel shows analysis of the three types of RNA using probe 1 (from the 7 VMP gene); this probe hybridizes to one major band that is present only in the 7 strain. Similarly, the probe from the 21 gene (2) hybridizes to a band of approximately the same size (1,100 bases) that is present only in the 21 strain. This result shows that the difference between the two strains in VMP expression is indeed at the transcriptional level, and that the probes used are indeed specific for the different VMP genes.

Methods. The experiments were done as follows: total *Borrelia* RNA was isolated and electrophoresed as described¹⁰, transferred to a Biohyne Nylon membrane and baked under vacuum. After 1 h of prehybridization, the blot was incubated at 37 °C for 16 h in 6 \times SSC, 50% formamide, 1% SDS, 0.1 \times Denhardt's and 100 $\mu\text{g ml}^{-1}$ sonicated *E. coli* DNA and the probe. Probes were made by nick-translation of the subclone DNA, using 40 μCi of [α -³²P]dATP per reaction. Free nucleotides were removed by spinning the sample through a 200- μl BioRad P200 column. After hybridization the blots were rinsed at room temperature and washed in 2 \times SSC, 1% SDS at 50 °C for 30 min. Exposure was for between 2 and 16 h at -70 °C with an intensifying screen.



recombinant p21.8 expresses a truncated VMP 21. Similarly, the recombinant p7.1 expresses a truncated VMP 7 (not shown). Thus, the *Pst*I site interrupts the coding sequence of both VMP genes. This result also indicates the direction of reading of the VMP genes, as the 5' end of the gene is apparently contained within the subcloned fragment.

Activation of VMP genes

The RNA analysis showed that the switch from serotype 7 to serotype 21 involves switching-off VMP 7 transcription and switching-on VMP 21 transcription. We showed that expression of the VMP genes in *E. coli* is associated with juxtaposition to a specific DNA segment. If the exclusive expression of one VMP gene is the result of the presence of that VMP gene adjacent to the expression sequence, then the switch from serotype 7 to 21 should involve the replacement of the 7 VMP gene adjacent to the expression sequence by the 21 VMP gene. The probes specific for the 7 or 21 VMP genes (1 and 2) were used to follow the fate of the 7 and 21 VMP genes in the switch.

Figure 3A, panel b shows *Pst*I-digested DNA from 7, 21 and C cells, hybridized with probe 1. All three strains contain a fragment of 8.5 kbp that hybridizes with the probe. The same fragment is found in clone p7.12, which does not express VMP 7; this suggests that the 8.5-kbp fragment represents the silent form of the 7 VMP gene in all serotypes. The 7 DNA contains an additional fragment of 2.9 kbp that hybridizes with the probe; this is the fragment found in clone p7.16 (the VMP 7-expressing clone). The expressed copy of the 7 VMP gene is no longer present after the switch to the 21 serotype. When the VMP 21-specific probe (2) was used, a 1,250-base pair (bp) *Pst*I fragment was observed in the three serotypes (Fig. 3A, panel c); this is the fragment present in clone p7.31 which contains the silent 21 VMP gene. In addition to this fragment, the 21 DNA contains a 2.8-kbp fragment that hybridizes with the probe, and this is

the fragment that is found in the VMP 21-expressing clone in which the 21 VMP gene is fused to the expression site. This result shows that the activation of the 21 VMP gene is also associated with a duplication of the gene and fusion to the expression sequence.

The above results suggest that in each serotype the expression sequence is fused to a different VMP gene. To demonstrate this, we used the expression sequence (fragment 3, Fig. 1B) as a probe in a Southern blot analysis; the fragments specific for the expressed 7 VMP gene and the expressed 21 VMP gene showed hybridization (2.9 and 2.8 kbp respectively; see Fig. 3A, panel a). In the C serotype the expression sequence is fused to yet another DNA segment (resulting in a 3.9-kbp *Pst*I segment). In addition, probe 3 hybridized to a 4.7-kbp fragment common to all serotypes (see legend to Fig. 3A).

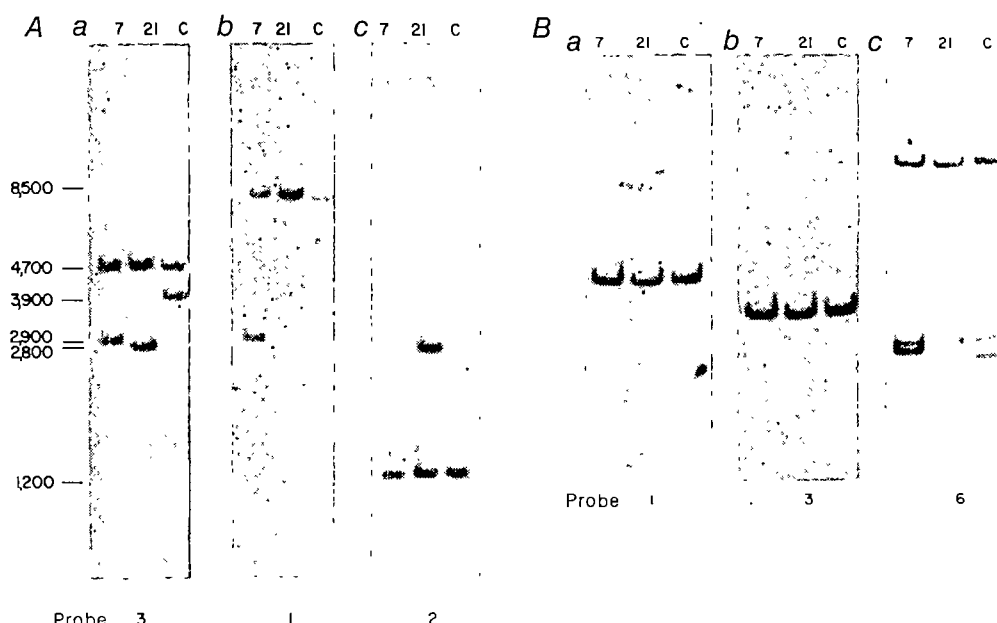
Several conclusions can be drawn from these data: in the 7 serotypes two copies of the entire 7 VMP gene are found. One of these copies is the transcribed copy, and this is fused to the expression sequence. In addition, the cells contain an untranscribed copy of the 21 VMP gene. When the cells switch to serotype 21, the copy of the 7 VMP gene that is adjacent to the expression site is replaced by a copy of the 21 VMP gene.

The site at which the expression sequence and the VMP gene are fused can be referred to as the recombination site. In the expressed VMP clone this site was mapped as follows: the 2.9-kbp *Pst*I fragment containing the site was divided into *Hind*III fragments. Each of these fragments was used as a probe of *Hind*III-digested DNA from different serotypes. The *Hind*III fragment containing the recombination site should show a pattern that is different in the three strains. As shown in Fig. 3B, the fragment numbered 6 contains the recombination site. No other rearrangement was seen in this 2.9-kbp *Pst*I fragment.

We have shown that the activation of a VMP gene is the result of fusion of that gene to the expression site. We do not know

Fig. 3 **A**, Southern analysis of *Pst*I-digested DNA from the three serotypes 7, 21 and C. The probes were 3(a), 1(b) and 2(c). Probe 1 hybridizes to an additional band of 2.9 kbp in the 7 DNA; probe 2 hybridizes to an additional 2.8-kbp fragment in the 21 DNA. Note that probe 3 hybridizes to a 2.9-kbp fragment in the 7 DNA and also a 2.8-kbp band in the 21 DNA, as well as to a 3.9-kbp fragment in the C DNA. All three of these fragments are specific for each serotype. In addition, probe 3 hybridizes to a fragment of 4.7 kbp that is present in all three serotypes; this *Pst*I fragment has been mapped. The plasmids p7.7 and p21.4 (that is, the plasmids containing the expression site) contain a 4.7-kbp *Pst*I fragment that hybridizes with probe 3 (not shown). This *Pst*I fragment was mapped adjacent to the *Pst*I fragment that contains the recombination site (see Fig. 1a). Nick-translation and hybridization

were done as described in Fig. 2 legend, except that hybridization was done at 42 °C, and the final washing was at 65 °C in 0.1×SSC, 1% SDS. The film was exposed for 2 h at -70 °C. **B**, Southern blot analysis of *Borrelia* DNA of three serotypes (7, 21 and C) digested with *Hind*III. **a**, The hybridization pattern obtained when subclone 1 was used as a probe (see Fig. 1). **b**, Same blot as **a** after removal of the first probe and hybridization with probe 3. **c**, Same blot again, but after hybridization with probe 6. The pattern using probes 1 and 3 is identical in all three lanes; that using probe 6 is different in each lane.



how the expression site activates the adjacent VMP gene; one possible explanation is that it contains a promoter for transcription reading into the adjacent DNA.

VMP genes carried on linear plasmids

We obtained an unusually high frequency of positive clones (5–10%) in the 7 and 21 phage banks, using probes that on Southern analysis of digested total DNA hybridized to only one or a few restriction fragments. If these genes were represented only once per genome this would predict an unusually small genome size. The most likely explanations are either that the genes are present in multiple copies in the chromosome of *B. hermsii* or that they are present on plasmids with a high copy number per cell. To resolve this issue we ran the total DNA on 0.5% agarose gels and observed several discrete bands. A better and quicker separation was achieved by pulse-field electrophoresis¹². The resolved bands were relatively small (~30 kbp) and optimal separation was obtained using short pulse times (1 s). Figure 4A shows total DNA from three *Borrelia* serotypes separated by pulse-field gel electrophoresis. The top band is the *Borrelia* chromosome (as a reference we included DNA from *E. coli* that was isolated using the same lysis and purification procedure; it co-migrated with the *Borrelia* chromosome). The banding patterns for serotypes 7 and 21 are very similar. Note, however, that the structure of the DNA of the C serotype is different—an extra band is visible. The fact that these molecules can be separated by pulse-field electrophoresis suggests that they are linear DNA molecules, as this technique does not resolve circular DNA well¹² (see below). The presence of extrachromosomal DNA does not seem to be unique to *B. hermsii*: as shown in Fig. 4A, the species *Borrelia burgdorferi*, the cause of Lyme disease, contains numerous bands. *E. coli* K-12 (Fig. 4A) and *Spirochaeta aurantia*, a free-living non-pathogenic spirochaete (not shown), yielded no detectable extrachromosomal DNA in this experiment. The simplest explanation for these bands is that they represent discrete extrachromosomal elements, that is, plasmids.

To determine whether these DNA elements contain the silent and expressed VMP genes, we probed the DNA separated by electrophoresis on a 0.7% agarose gel with probes for the 7 and

21 VMP genes and the expression site. As shown in Fig. 4B, the expression site lies on an extrachromosomal element of ~28 kbp in both the 7 and 21 strains. The VMP 7-specific probe hybridized to the 32-kbp plasmid in the 21 strain. Thus, the silent 7 VMP gene lies on a plasmid other than the one carrying the expression site. In the 7 strain two plasmids hybridized with the VMP 7 probe: one (32 kbp) containing the silent 7 VMP gene and the other (28 kbp) containing the expressed 7 VMP gene. In the 7 DNA the 28-kbp band hybridized with the VMP 2-specific probe. This result means that the silent 21 VMP gene is carried on a plasmid of the same size as the plasmid that carries the expression site. A clone of approximately 15 kbp containing the silent 21 gene (p7.31) contains no homology with clones of the expressed 7 gene (p7.7 and p7.16), which indicates that the silent 21 gene lies on a DNA molecule of 28 kbp that is different from the expression plasmid. This pattern shows that in the switch from type 7 to 21, the 7 VMP gene is removed from the 28-kbp plasmid, and a copy of the 21 VMP gene is inserted into the same site on the 28-kbp plasmid.

The behaviour of the extrachromosomal elements in pulse-field electrophoresis suggested that they might be linear rather than circular DNA molecules. We wanted to test whether the DNA of 28 kbp was indeed linear, and if so, whether the sequences on the molecule were circularly permuted. Therefore, we treated the putative ends of the DNA molecule with an exonuclease. If the DNA is linear and has specific ends, treatment with an exonuclease followed by restriction enzyme digestion and gel electrophoresis should result in a shift in the relative molecular mass (M_r) of specific restriction fragments. As we could obtain only small amounts of the 28-kbp DNA, we did the experiment as follows: total DNA from the 7 serotype was digested for different lengths of time with *Bal*31 nuclease. The DNA was then digested with *Pst*I and separated on a gel. The fragments contained in the 28-kbp band were visualized by Southern blotting and hybridization with nick-translated 28-kbp plasmid DNA. The result is shown in Fig. 5A: the probe hybridized to a series of *Pst*I fragments in the 7 DNA, but only one of these bands showed a shift down of M_r after treatment with *Bal*31 nuclease. Once this band had been degraded, a second band began to shift down. We concluded from this experiment

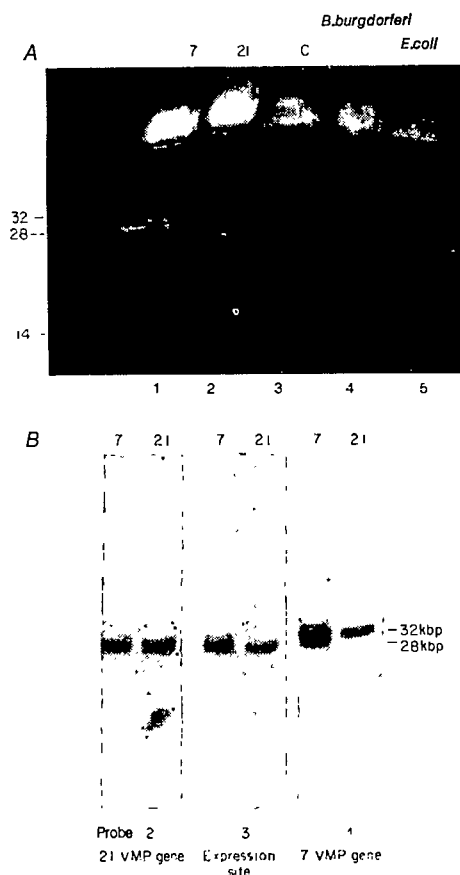


Fig. 4 **A**, Pulse-field gel electrophoresis of *Borrelia* DNA. DNA from *B. hermsii* and *E. coli* was isolated as described¹⁰ and separated as follows: a 1.1% agarose gel was placed in a tray with two pairs of electrodes (a slight modification of the original method described by Schwartz and Cantor¹², Stanford workshop); 10 μ l of DNA was loaded per lane and electrophoresis was at 175 V for 16 h, with the field changing every second. The gel was stained with ethidium bromide and photographed as any DNA/agarose gel. The top band is the chromosomal DNA (in lane 5, *E. coli* chromosomal DNA is shown). In addition, there are several extrachromosomal DNA elements visible. The pulse time (1 s) was chosen to optimize separation of the 28-kbp band from the 32-kbp band. When longer times are chosen the larger elements are separated better, and it is then clear that the band just beneath the chromosomal band is indeed separate. **B**, Southern analysis of the extrachromosomal *Borrelia* DNA. Total *Borrelia* DNA from the 7 and 21 serotypes was separated on a 0.7% agarose gel (25 V, 60 h at 4 °C). The resulting pattern is identical to that shown in **A**. We chose standard separation in a low-percentage gel rather than in a high-percentage gel in pulse-field electrophoresis because the capillary transfer of large molecules is better from a low-percentage gel. However, the experiment has also been done after transfer from a pulse-field gel, or by hybridizing to the dried gel itself, with similar results. The gel was treated briefly with 0.25 M HCl, then the DNA was transferred as described above and hybridized with the probes as indicated. The same blot was used with all three probes.

that the 28-kbp band results from a linear DNA molecule having specific ends. We do not yet know in what form this molecule exists in the cell. It seems probable, however, that the ends of the DNA molecule are held together by other macromolecules in the cell (for example, proteins). As only one restriction fragment shifted in the gel in Fig. 5A, it seems that one of the ends of the plasmid was protected from degradation, for example by an amino acid or peptide that was covalently bound and was not removed by the protease and phenol treatment during the DNA purification. We have used nick-translated restriction fragments instead of the total 28-kbp DNA as probes for similar gels. Using the expression-linked 21 VMP gene as a probe (probe 2) with *Bal31* digestion of serotype 21 DNA, it was shown that this fragment is degraded late in the time series (see Fig. 5B),

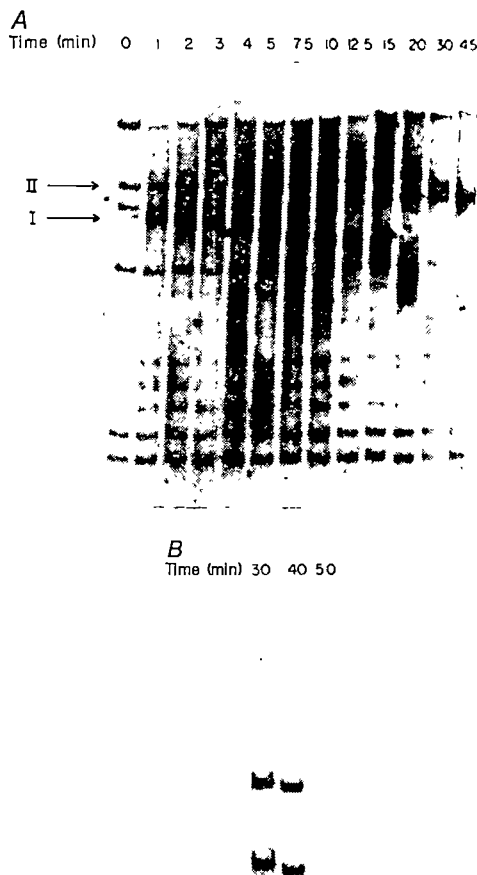


Fig. 5 *Bal31* degradation of the terminal region of the 28-kbp plasmid. **A**, *Borrelia* DNA (serotype 7) was treated for the indicated time with 0.15 units of *Bal31* (Boehringer Mannheim). EDTA was added to a final concentration of 20 mM, then the DNA was recovered by alcohol precipitation and digested with *Pst*I before separation on a 1% agarose gel. After blotting the gel was hybridized with '28-kbp probe'. This probe was obtained as follows: serotype 7 DNA was run out on a pulse-field gel, and after ethidium bromide staining the 28-kbp plasmid band was cut out of the gel, and the DNA recovered from it. Then it was nick-translated as described above. The arrow labelled I indicates the first band that disappears; the arrow labelled II indicates the second band. **B**, 21 DNA was treated in a time series with *Bal31* as described for **A**. As a probe we used clone 2 (see Fig. 1B), which is a restriction fragment internal to the 21 VMP gene. The two bands that are seen are the 1,250- and 2,800-bp fragments observed in Fig. 3A, corresponding to the silent and the expressed 21 gene respectively. The fragments were initially not affected during the time series. The timepoints are shown at which suddenly both bands began to shift down simultaneously.

hence the transcribed 21 VMP gene is located several kilobases away from the nuclease-sensitive end on a linear plasmid. Note that the bands corresponding to the silent and expressed 21 VMP genes shifted down at the same time; they are therefore at approximately the same distance from the *Bal31*-sensitive end of the plasmids, so the distance from the 21 VMP gene to the end of the plasmid downstream of the gene is not significantly changed by the recombination of the silent gene into the expression site.

Switch mechanism

A schematic representation of the molecular basis of antigenic variation is given in Fig. 6. We have shown for two VMP genes that expression results from the presence of a copy of the gene

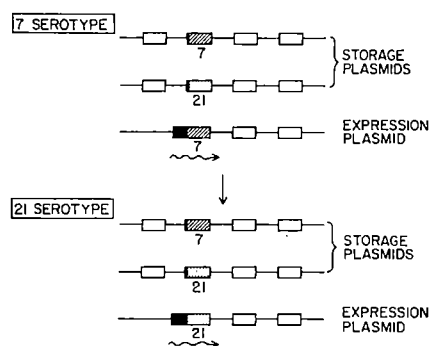


Fig. 6 Model for the molecular basis of antigenic variation in *Borrelia*. The relevant plasmids of the 7 and 21 serotypes are shown. The storage plasmids are identical in both strains. The 21 VMP gene in the 7 cells is not transcribed. It transposes into the expression site, and the resident 7 VMP gene is removed. The VMP gene that is adjacent to the expression sequence is transcribed. As discussed in the text, several mechanisms for the recombination reaction are possible, but we favour the one in which a reciprocal site-specific recombination is followed by loss of one of the two reaction products.

in the expression site on the 28-kbp plasmid. When the serotype switches from 7 to 21, the copy of the 7 VMP gene in the expression site is removed and replaced by a copy of the 21 VMP gene. The silent copies of the 7 and 21 VMP genes seem unaffected by the switch.

There are three possible mechanisms for the actual recombination reaction: (1) The switch could be the result of a single reciprocal site-specific recombination reaction with subsequent loss of one of the recombination products. (2) The silent genes could lie on 'cassettes' which can be activated by a duplicative transposition. This would then involve a gene conversion event or two cross-overs at either end of the cassette. (3) The silent VMP genes could be activated by insertion of a transposable DNA element upstream of the genes. Model (2) would predict that, in addition to the recombination site that we described, there would be a second recombination site at the other end of the cassette. In model (3) the second site would be at the other end of the inserted transposable element. We have found no such sites. The restriction maps upstream of the expressed 7 and 21 genes are completely colinear for at least 10 kbp, which makes model (3) unlikely. The sequences 3' of the expressed 21 gene (the 2 kbp present in p21.4) show no homology to those 3' of the expressed 7 gene in p7.16, which makes model (2) unlikely. As the plasmids are linear, it is conceivable that the recombination reaction responsible for the switch is a single site-specific cross-over between two linear plasmids. The fact that the approximate distance from the 21 VMP gene to the downstream end of the plasmid is unchanged after the switch is compatible with this idea. On the basis of present evidence, model (1), the single site-specific recombination event, seems the most likely.

The net result of the recombination reaction that fuses a new VMP gene to the expression site will initially be that the cell contains both types of expression plasmids (assuming that only one plasmid switches at a time). For example, in the case described here the plasmid containing the expressed 7 VMP gene could recombine with the plasmid containing the silent 21 gene. The products are the 21 expression plasmid, and another DNA molecule that either segregates out or is actively removed. The cell then contains both type 7 and type 21 expression plasmids. The immune system presumably selects for the segregants that no longer contain the 7 expression plasmid. A prediction following from this model is that cells that express two VMPs at the same time should be detectable *in vitro*. This prediction is now being tested.

Serotype C is different from the serotypes recovered from spirochaetaemic mice. VMP C is much smaller than the VMPs found *in vivo*⁵. No antigenic switching of serotype C cells has

been observed in culture medium, and no relapses have been noted after infection of a mouse with serotype C cells (A.G.B., unpublished observations). As shown, serotype C DNA contains an extra element; it is this DNA segment that contains the expression site for the 7 and 21 VMP genes, and this sequence is fused to yet another sequence in the C strain (Fig. 3A). At present it is unknown whether the sequence is indeed fused to the expressed C VMP gene. The site may be 'empty' and the expressed C gene may be found in another expression site.

We chose to analyse one switch in detail, but it is not necessarily true that the other 24 antigenic types thus far found after infection are all switched on by recombination of the corresponding VMP gene into the same site. A similar analysis to that described here of cell lines of other serotypes will show whether this is the case.

Genetic switches in other organisms

There exist several other examples of DNA rearrangements in prokaryotes that are responsible for switches in gene expression¹³⁻¹⁵. The mechanism of gene activation by recombination of silent genes into an expression site is known in eukaryotes. A binary switch exists in yeast, where the mating type varies by transposition of a silent gene into the *MAT* locus¹⁶. The protozoan *Trypanosoma brucei* can vary its antigens by a mechanism that is very similar to the one described here¹. The parallels between the bacterial and the eukaryotic infectious microorganisms *B. hermsii* and *T. brucei* are striking: the function of the switch (to escape the host immune system), the mechanism of the switch (recombination into an expression site on minichromosomes) and the ordered appearance of the different serotypes are found in both cases^{1,4,5}. It is tempting to speculate on an evolutionary relationship between the two microorganisms. A more likely alternative, however, is that the strong and very direct selective pressure provided by the immune system has resulted in convergence of these two blood-borne and vector-transmitted microorganisms. DNA rearrangements provide the immune system with a means of generating antibodies against a large number of infections; this finds its complement in a system of DNA rearrangements in the microorganism that is designed to escape the immune system.

Linear plasmids and pathogenicity

We have shown that the silent and expressed genes for two surface proteins of *B. hermsii* lie on linear extrachromosomal elements, based on the following evidence. The high frequency with which clones carrying specific VMP genes are found in gene banks shows that these genes must be present many times in the genome. On low-percentage agarose gels and in pulse-field gels, there occur discrete bands of DNA that are not covalently linked to the chromosome. Probes specific for the VMP genes hybridize to these extrachromosomal DNA elements (and not to the chromosome). The DNA molecules carrying the VMP genes have specific ends that are sensitive to degradation by *Bal*31 nuclease.

Linear genetic elements have been found previously in prokaryotic cells, but usually they are only transiently linear (for example, several bacteriophages on infection). The DNA of *Bacillus* phage ϕ 29 exists in linear form in the cell and has proteins attached to its ends¹⁷. Linear DNA has been observed in yeast *Streptomyces* sp.¹⁸ and in the mitochondria of a number of Protozoa¹⁹. In *B. hermsii* we detected no circular DNA that hybridizes with the probes specific for the VMP genes, therefore the linear form is probably the replicating form of the plasmid. (Circular plasmid DNA can be isolated from *Borrelia*, but this DNA did not hybridize with the VMP-specific probes; J. Meier and A.G.B., unpublished.) We have demonstrated that after deproteination the DNA molecules are linear, but we do not yet know how the molecules exist in the cell. The fact that only one of the two ends of the molecule seems sensitive to *Bal*31 nuclease could mean that there is a covalent modification at one end of the molecule.

Linear plasmids have not been observed previously in pathogenic microorganisms. However, the usual techniques for separating extrachromosomal DNA from the chromosome are largely unsuitable for detecting linear plasmids. On CsCl ethidium bromide gradients, linear extrachromosomal DNA cannot necessarily be separated from the chromosomal DNA. Several other methods for isolation of plasmid DNA allow isolation of only relatively small DNA molecules. There are clear advantages for an infectious microorganism in having a battery of silent and expressed surface protein genes on extrachromosomal DNA: the copy number of the expression plasmid can be high (which facilitates high levels of expression), and

rearrangements can occur at high frequency to activate silent genes, without affecting the stability of the chromosome. An extensive search of other pathogenic microorganisms (and especially those that have a large repertoire of variable antigens) using the technique of pulse-field electrophoresis may reveal an important role of linear extrachromosomal DNA in pathogenesis.

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A cDNA clone encoding an *S*-locus-specific glycoprotein from *Brassica oleracea*

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The pollen-stigma interaction resulting in self-incompatibility in the cruciferous plant Brassica has been correlated with glycoproteins that display polymorphisms in all S genotypes examined. A complementary DNA clone encoding these glycoproteins has been isolated and characterized. Polymorphisms in homologous restriction fragments of Brassica genomic DNA segregate precisely with alleles at the S locus.

IN many species representing very diverse angiosperm families, self-fertilization is blocked by a mechanism termed self-incompatibility¹. Following self-pollination, interaction between the stigma or style and pollen elicits a defined morphological response preventing normal pollen tube growth; fertilization is prevented because these pollen tubes are unable to penetrate the full length of the stigma and style. The specificity of the incompatible interaction is determined by one or more genetic loci, and requires that the alleles carried by the male and female parents are identical. In the crucifer *Brassica*, the self-incompatible reaction is localized at the stigma surface, and occurs within minutes after the initial contact between the pollen and the papillar cells on the outer surface of the stigma. In this genus, self-incompatibility is under the control of a single genetic locus, the *S* locus, which is highly polymorphic, some 50 alleles having been identified^{2,3}.

A molecular analysis of the genetic control of incompatibility may best be performed by detecting antigens specific to various *S*-locus alleles in stigma homogenates from different *Brassica* strains⁴. These antigens have been shown to correspond to glycoproteins that may be resolved in various electrophoretic systems⁵⁻⁸. Several lines of evidence suggest that these glycoproteins have an important role in incompatibility. (1) The mobilities of these molecules vary in stigma extracts derived from *Brassica* strains with different *S*-locus alleles⁸. (2) These molecules are found in the stigma but not in style or seedling tissue⁹. (3) The increased rate of synthesis of these *S*-locus-specific glycoproteins (SLSGs) in the developing stigma correlates with the onset of the incompatibility reaction in the stigma⁹. (4) Mutations in genes unlinked to the *S* locus which result in self-compatibility are also associated with reduced levels of these

molecules¹⁰. (5) Most importantly, the inheritance of the various forms of SLSG correlates perfectly with the segregation of *S* alleles in genetic crosses, indicating that the gene responsible for this polymorphism must be genetically located at or extremely closely linked to the *S* locus^{6,11}.

Here we report the isolation of a complementary DNA clone containing sequences encoding an *S*-locus-specific glycoprotein from *Brassica oleracea*. We show that the spatial and temporal distribution of the messenger RNA homologous to these sequences mirrors the appearance of the *S*-locus-specific glycoprotein. Several fragments of *B. oleracea* genomic DNA, generated by restriction endonucleases, hybridize with the SLSG cDNA clone; polymorphisms in certain of these fragments segregate precisely with alleles at the *S* locus.

Isolation

Our strategy for isolating SLSG-encoding DNA sequences was based on the finding that, in some *Brassica* strains, ~5% of the protein synthesis in the self-incompatible bud stigma is devoted to the SLSG synthesis⁹. Thus, it seemed likely that a high proportion of clones in a cDNA library constructed with poly(A)⁺ RNA from mature, incompatible stigmas would contain SLSG sequences. It had also been observed previously that SLSG molecules appear in the stigma but not in leaf or seedling tissue⁸. We thus expected that in such a library SLSG cDNA clones would be enriched in the fraction of clones that would hybridize strongly to radioactive cDNA¹² corresponding to mature stigma mRNA, but not to labelled leaf or seedling-specific cDNA. Poly(A)⁺ RNA from incompatible stigmas was therefore isolated and used as a template for a cDNA library constructed in the plasmid pBR322 using *Bam*HI linkers¹³.

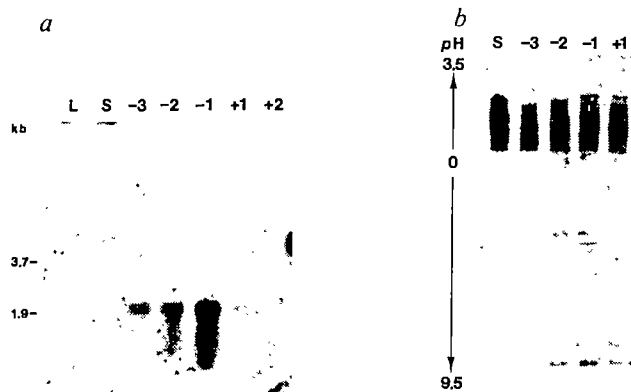


Fig. 1 Temporal regulation of pBOS5-specific RNA and of SLGG expression in the S_6S_6 homozygote. *a*, Northern analysis of RNA from *Brassica* tissues. L, leaf tissue; S, stylar tissue; -3, -2, -1, +1 and +2 refer to stigma developmental stages in days before or after anthesis. *b*, Synthesis of SLGG in developing stigmas. The origin of isoelectric focusing 'O' on the autoradiograph and the generated pH gradient are indicated. The arrow indicates the S_6 -specific SLGG band. Lane designations as in *a*.

Methods. Each inflorescence was divided into developmental zones designated -3, -2, -1, +1 and +2, each consisting of a sequence of three flowers or flower buds. Stigmas from the -3, -2 and -1 zones were stigmas derived from immature flower buds that would normally reach anthesis in 3, 2 and 1 days, respectively; stigmas from the +1 and the +2 zones were mature stigmas from flowers at 1 and 2 days past anthesis, respectively. Stigmas from the -3 and -2 zones are self-compatible, while those from the remaining zones are self-incompatible. *a*, RNA was isolated from *Brassica* tissues using an SDS-proteinase K extraction procedure^{21,22}. Samples containing 1 μ g of denatured RNA were electrophoresed on 1% (w/v) agarose gels in the presence of formaldehyde¹³ and transferred to nitrocellulose²³. Parallel untransferred lanes were stained with ethidium bromide and the location of the ribosomal RNA bands determined. Blots were prehybridized at 42 °C in 50% formamide (w/v), 1% Denhardt's solution, 0.2% SDS (w/v), 0.9 M NaCl, 50 mM NaH_2PO_4 pH 7.4, 5 mM EDTA and 100 μ g ml^{-1} carrier calf thymus DNA. The filters were hybridized to nick-translated pBOS5 DNA²⁴, washed and exposed to Kodak XAR-5 film. *b*, Excised stigmas and 1-mm sections of stylar tissue were metabolically labelled by submersion in a ^{14}C -amino-acid mixture as described elsewhere⁹. Isoelectric focusing analysis of radioactively labelled proteins and autoradiography were also as previously described⁹.

Approximately 2,000 independent clones were picked randomly from this cDNA library, and DNA from these colonies was then transferred to each of two nitrocellulose filters and hybridized with the appropriate probes¹⁴. Eight colonies were found to hybridize strongly with stigma-derived cDNA probe but not with seedling cDNA probe. Plasmid DNA was prepared from each of these eight colonies, and digests with the restriction endonuclease *Bam*HI were performed such that the cDNA inserts could be separated from the plasmid vector by gel electrophoresis. Cross-hybridization experiments performed on these digests indicated that the *Brassica* DNAs in all eight recombinant plasmids were homologous (results not shown). The plasmid containing the largest cDNA insertion, pBOS5, was chosen for use in all subsequent experiments reported here.

pBOS5 expression during development

To examine more directly the proposition that the inserts in plasmid pBOS5 and its relatives correspond to SLGG mRNA, the concentration of pBOS5-homologous mRNA was measured as a function of stigma development (Fig. 1). Northern analysis of RNA prepared from populations of staged stigmas reveals a prominent band ~2 kilobases (kb) long which cannot be detected in leaf and style tissue (Fig. 1*a*). This RNA species must be quite abundant in the stigma, as intense signals are obtained even when poly(A)⁺ RNA has not been purified. The pattern of expression of these sequences parallels the pattern of synthesis

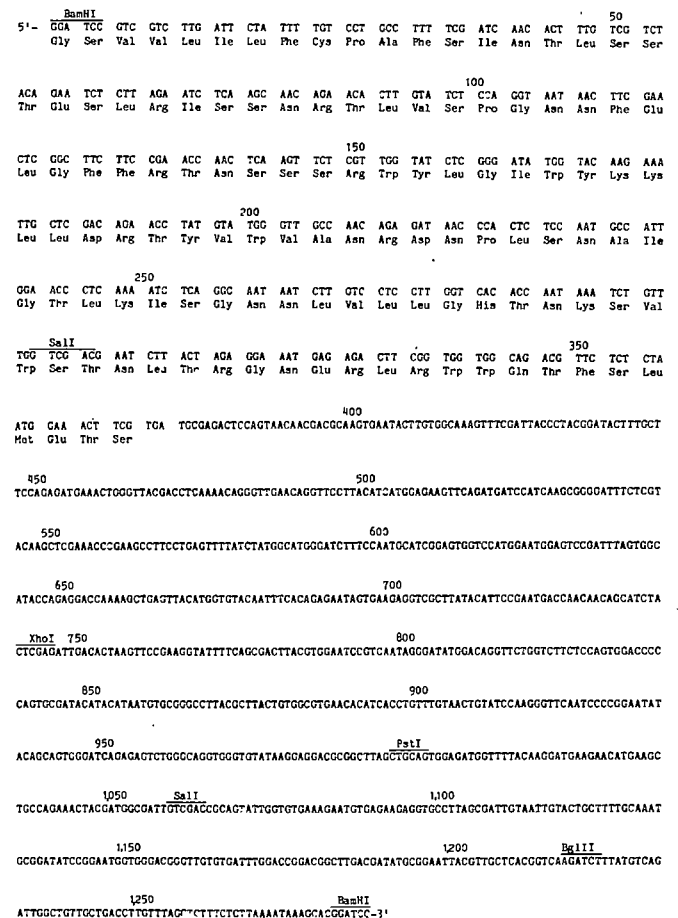


Fig. 2 Nucleotide sequence and deduced amino-acid sequence of the cDNA insert in pBOS5. Sequences delineated by the indicated restriction sites were subcloned into the M13 vectors M13 mp10 and M13 mp11 (ref. 25). Sequencing was carried out by the dideoxynucleotide chain-termination method¹⁵ using a synthetic 17-mer as primer (New England Biolabs).

of SLGG in the stigma (Fig. 1*b*), a maximum level being observed 1 day before the flower opens. It should be emphasized that the stigmas of immature buds are self-compatible, so that fertilization with pollen which would normally induce an incompatible response is possible by mechanically opening the bud and placing such pollen directly on the stigma. As the buds develop, the stigmas become self-incompatible 1 day before anthesis, correlating with the observed peak concentration of pBOS5-homologous RNA.

An S-locus-specific glycoprotein

Although the previous results were consistent with the notion that pBOS5 contains sequences encoding SLGG, further tests were required to confirm the identity of this clone. Thus, the nucleotide sequence of the cDNA insert in pBOS5 was determined to guide strategies for the construction of chimaeric genes in which *Brassica* DNA could be transcribed and translated in *Escherichia coli* cells, in order to establish whether the resultant fusion protein would react with antiserum to authentic SLGG. Restriction endonuclease-generated fragments of the pBOS5 insert were subcloned into the M13 vectors mp10 and mp11 and the resulting single-stranded DNAs subjected to sequence analysis by the Sanger technique¹⁵.

Figure 2 shows the results of the nucleotide sequence determination. Although five of the six possible reading frames are blocked by multiple nonsense codons throughout the entire 1,284-base pair (bp) insert, the remaining reading frame translates into a polypeptide of 123 amino acids. The nucleotide sequence AATAAA, which has been implicated as a signal for the polyadenylation of mRNA¹⁶, is located downstream of the putative SLGG coding region, 11–16 bp from the end of the

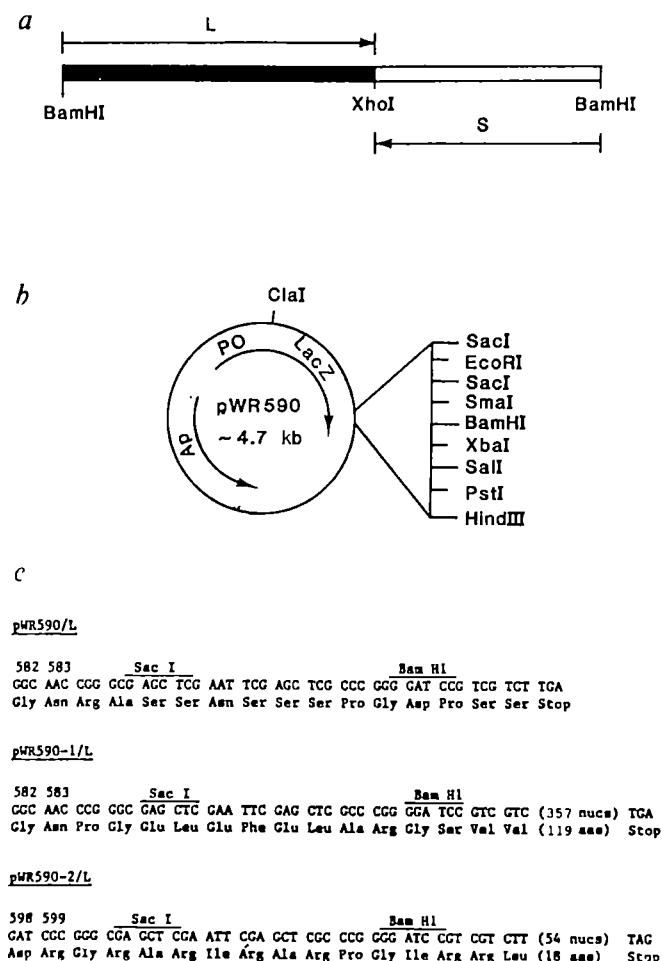


Fig. 3 Subcloning of the pBOS5 cDNA insert into the pWR590 series of expression vectors. **a**, Generation of the pBOS5 L and S fragments by *Bam*HI/*Xho*I digestion. Arrows indicate the resulting direction of transcription in the chimaeric plasmid. **b**, Map of pWR590. Arrows indicate the direction of transcription. *lacZ'* denotes a shortened *lacZ* gene encoding about 590 amino acids. The expanded region following the *lacZ'* gene contains the poly-linker sequence. **c**, Nucleotide sequences of the expression vectors at the fusion junctions, and the expected translational products. nucs, nucleotides; aas, amino acids.

Methods. To construct expression plasmids of the *Brassica* cDNA sequence, the 745-bp (L) and 538-bp (S) *Bam*HI/*Xho*I fragments of pBOS5 were isolated from a 1% low-melting agarose gel and ligated to *Bam*HI and *Sac*I digests of pWR590, pWR590-1 and pWR590-2. The resulting six plasmid constructions, designated pWR590-L, pWR590-1L, pWR590-2L, pWR590-S, pWR590-1S and pWR590-2S, were used to transform *E. coli* SF8 cells²⁶.

cDNA insert presumed to have been derived from the 3' end of the mRNA. Such an interpretation of the sequence data implies that the 3'-noncoding region of the messenger is ~900 nucleotides long. Although such an extensive 3'-noncoding region is unusual, precedents have been noted in other eukaryotic mRNAs¹⁷. The absence of a poly(A) tract may be explained by the finding that the *Bam*HI sequences flanking both sides of the cDNA insert do not resemble the *Bam*HI linker sequence used to construct the cDNA clone. We presume that a *Bam*HI recognition site is located in the full-length cDNA sequence between the polyadenylation signal and the poly(A) tail of the messenger, such that this *Bam*HI site would be inserted into the pBR322 vector in place of the end provided by the linker sequence. Our inability to obtain clones with longer cDNA sequences is similarly explained by the presence of at least one additional *Bam*HI site in sequences internal to the mRNA.

Although incomplete, the predicted polypeptide has properties consistent with its corresponding to SLSG. Approximately 11% of the amino acids are basic and only 5% are acidic, in

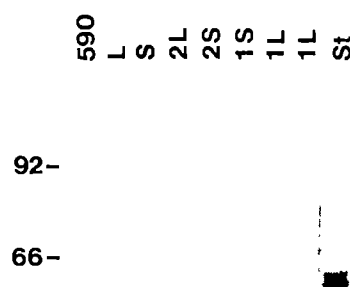


Fig. 4 Western blot analysis of fusion proteins encoded by constructs in the pWR590 series of expression vectors. 'St' designates the lane with a stigma extract; the characteristic M_r heterogeneity⁸ of SLSG is shown. Designations above the other lanes indicate extracts from *E. coli* cells transformed with the corresponding pWR590 constructs. One band corresponding to the antigenic fusion protein is seen in lanes 1L. The mobilities of M_r standards are shown ($\times 10^{-3}$).

Methods. Transformed *E. coli* SF8 strains were grown overnight in the presence of 1 mM isopropyl thiogalactoside. Extracts were prepared by sonication in 7 M guanidine hydrochloride⁹, and aliquots equivalent to 500 μ l of culture were subjected to electrophoresis on 10% SDS acrylamide gels²⁷. The separated proteins were transferred electrophoretically to nitrocellulose membrane²⁸. Immunolabelling of nitrocellulose blots was as described elsewhere²⁹. The first-stage antiserum was raised in a New Zealand White rabbit by multiple intradermal injections of purified S_6 -specific glycoprotein. The second-stage probe was peroxidase-conjugated protein A.

keeping with the basic nature of most SLSG molecules analysed to date. Four potential *N*-glycosylation sites which follow the general rule of Asn-X-Thr/Ser (ref. 18) can be identified beginning at asparagine residues 28, 46, 96 and 103. Many serine and threonine residues are also potentially available for *O*-glycosylation.

The presumptive coding region of the cDNA insert is entirely contained within a fragment of DNA (fragment L in Fig. 3a) demarcated by recognition sites for the enzymes *Bam*HI (at positions 1-6) and *Xho*I (nucleotides 740-745). A family of three expression vectors (pWR590, pWR590-1 and pWR590-2) has recently been described in which foreign DNA may be inserted in all three possible translational reading frames into a polylinker region such that these exogenous sequences can be translated by *E. coli* cells into a fusion protein, the first 590 amino acids of which are supplied by a truncated *E. coli* β -galactosidase gene (ref. 19 and Fig. 3b). As illustrated in Fig. 3c, fragment L from pBOS5 theoretically would encode a fusion protein containing determinants encoded by the long open reading frame predicted by the DNA sequence data only when inserted into the vector pWR590-1.

Fragment L and fragment S, a *Bam*HI/*Xho*I fragment containing the remainder of the *Brassica* insert (Fig. 3a), were cloned into the pWR590 vectors such that all six possible reading frames of the *Brassica* cDNA could be assayed for their ability to promote the expression in *E. coli* cells of polypeptide determinants which could react with antiserum to authentic SLSG glycoprotein. Extracts prepared from cultures of *E. coli* transformed with the six constructs were probed with antibodies to SLSG (Fig. 4). It may be seen that in lanes 1L (pWR590-1L, see Fig. 3c), a band of relative molecular mass (M_r) ~85,000 reacts with the antiserum. As predicted, this band is not found in lysates from cells containing any of the other five chimaeric plasmids or the parental pWR590 vector. Furthermore, the size

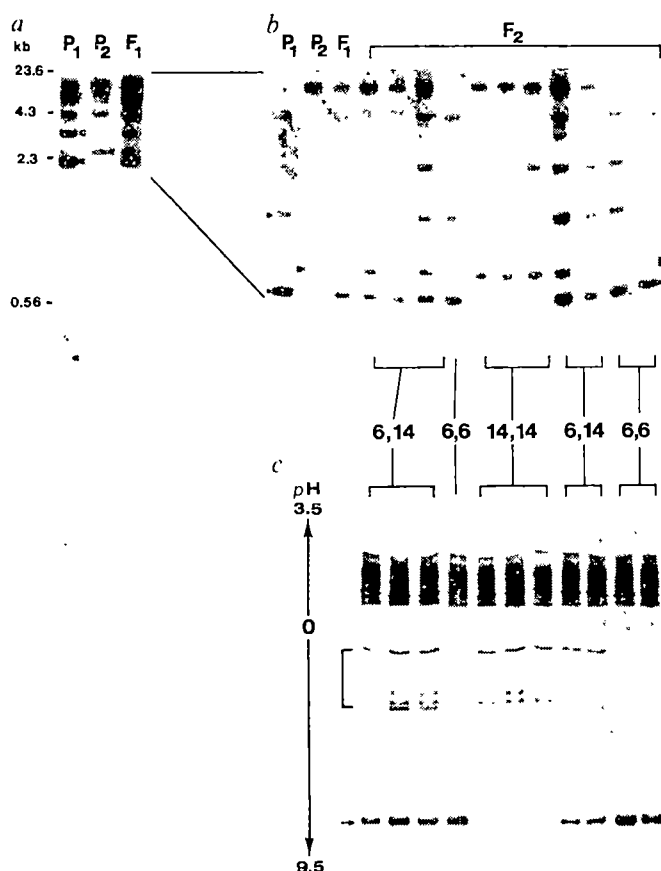


Fig. 5 Analysis of a population of plants segregating for the S_6 and S_{14} alleles. *a*, Southern analysis of *Bam*HI/*Pst*I digests of genomic DNA from the S_6 -homozygous parent (P_1), the S_{14} -homozygous parent (P_2) and the heterozygous F_1 hybrid. Arrowheads indicate representative restriction fragments that segregate in correlation with the S_6 and S_{14} alleles. The nick-translated cDNA insert of pBOS5 was the probe. *b*, Southern analysis of *Bam*HI/*Pst*I digests of DNA from P_1 , P_2 , F_1 and an F_2 population derived by self-pollinating immature self-compatible stigmas of the F_1 heterozygotes. Electrophoresis was carried out longer than in *a* in order to resolve the higher- M_r restriction fragments. 6,6: S_6 homozygotes; 14,14: S_{14} homozygotes; 6,14: S_6S_{14} heterozygotes. The probe and arrowheads are as in *a*. *c*, Isoelectric focusing patterns of stigma extracts from the F_2 plants in *b*. The arrow indicates the S_6 -specific band and the bracket indicates the complex of bands characteristic of the S_{14} allele. The origin of electrophoresis (O) and the pH gradient are indicated. The gel was stained with Coomassie blue R250.

Methods. The S_6 and S_{14} homozygotes are inbreds maintained at Cornell. Incompatibility phenotypes were determined in F_2 plants by crossing to tester homozygous strains and by diallel analysis. The pollinated flower stigmas were stained with decolorized aniline blue and pollen-tube development monitored by fluorescence microscopy^{30,31}. DNA was prepared from ~0.25 g of leaf tissue by a modification of a rapid phage lysis protocol. Digests of genomic DNA were electrophoresed on 0.9% (w/v) agarose gels and transferred to GeneScreen filters (NEN)³². Filters were prehybridized and hybridized in 50% (w/v) dextran sulphate, 330 mM NaP_i, pH 7.0, 10 mM EDTA, 5% (w/v) SDS and 100 μ g ml⁻¹ carrier salmon testes DNA, at 67 °C. Washed filters were exposed to Kodak XAR-5.

of the prominent band is that expected for a fusion protein consisting of 593 amino acids from β -galactosidase and the polylinker region and 123 amino acids encoded by the *Brassica* cDNA clone.

Genomic pattern

Southern analysis of *Brassica* genomic DNA cleaved with a variety of restriction enzymes, including *Eco*RI, *Sal*I, *Xho*I, *Bam*HI and *Pst*I, revealed *S*-allele-associated restriction site polymorphisms. In each digest, several bands hybridized with

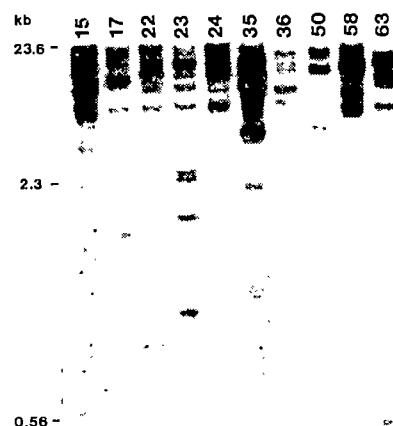


Fig. 6 Southern analysis of *Bam*HI/*Pst*I digests of DNA from different *S*-allele homozygotes of *Brassica*. The homozygous lines were obtained from the Gene Bank Facility at Wellesbourne, Warwick, UK, courtesy of Dr David Ockendon. The numbers above the lanes refer to the *S*-allele designations of the Gene Bank Facility. The probe is as in Fig. 5.

pBOS5. To determine whether these polymorphisms segregate with the *S* locus, DNA was prepared from an F_2 population obtained by the selfing of heterozygous F_1 plants derived from S_6S_6 and $S_{14}S_{14}$ homozygotes. Analysis of this DNA cleaved with *Bam*HI and *Pst*I showed that some pBOS5-homologous bands are common to all of the plants examined, but that others are clearly specific to the S_6 or S_{14} alleles (arrowheads in Fig. 5*a, b*). Heterozygous S_6S_{14} plants display a hybrid pattern. Similar results have been observed for 55 plants from this F_2 cross and for an additional 22 F_2 plants segregating for the S_6 and S_{13} alleles. In all cases, the restriction site polymorphisms revealed by the pBOS5 probe co-segregate with the *S*-locus genotype as determined by pollination analysis, and with the SLSG polymorphism as determined by isoelectric focusing. A prominent band with an isoelectric point of ~9.0 is present in S_6S_6 homozygotes (Fig. 5*c*), but is replaced by a series of bands only slightly basic in charge in homozygous $S_{14}S_{14}$ plants. Of the heterozygous plants examined, all synthesize both sets of SLSG molecules.

Finally, the restriction endonuclease-generated fragments of *Brassica* DNA homologous to pBOS5 in plants homozygous for each of 14 different *S*-locus alleles demonstrate patterns specific to each genotype (Fig. 6) and suggest a variability sufficient to account for the 50 or so *S*-locus alleles known in *Brassica* populations.

Discussion

Our results show that pBOS5, and presumably the seven other homologous cDNA clones we have isolated, contain sequences specifying an *S*-locus-specific glycoprotein (SLSG). This identity has been established directly by the demonstration that pBOS5 encodes SLSG-specific antigenic determinants. A chimaeric gene, in which the cDNA insert from pBOS5 is joined in-frame with the *lacZ* gene from *E. coli*, directs the synthesis in *E. coli* cells of a fusion polypeptide which reacts with antiserum against authentic SLSG. Furthermore, the characteristics of the mRNA from which pBOS5 was derived are consistent with a molecule capable of encoding SLSG. This mRNA species is found in stigma tissue, but cannot be detected by our methods in styles, leaf or seedling tissue. The concentration of this messenger varies as a function of the maturity of the stigma in parallel with the rate at which SLSG is synthesized. Finally, DNA sequencing studies suggest that the 2-kb RNA homologous with pBOS5 is of sufficient length to encode SLSG. Although

estimates of the relative molecular mass of SLSG are complicated by the fact that these macromolecules are heavily glycosylated²⁰, preliminary experiments using material in which the carbohydrate moieties have been chemically removed from SLSG suggest M_r of 40,000–50,000 (J.B.N., unpublished results). Accounting for the long 3'-noncoding region deduced from the sequence data, over 1,100 nucleotides of protein-coding information remains in the 2-kb mRNA, sufficient to encode a polypeptide of 45,000 M_r . Furthermore, the amino-acid composition of the predicted polypeptide is consistent with that reported for three SLSGs in *B. oleracea*²⁰.

As outlined above, several lines of evidence strongly suggest that SLSG is the product of the *S* locus. The clearest support is provided by the finding that polymorphisms in the SLSG molecule itself and restriction site polymorphisms in the vicinity of certain genomic DNA sequences homologous with pBOS5 co-segregate with *S*-locus alleles. DNA likely to encode SLSG is thus genetically at or very closely linked to the *S* locus. Even in the event of fortuitous linkage between SLSG-encoding sequences and *S*-locus sequences, the latter would therefore be accessible to molecular cloning by the use of chromosome walking procedures extending from pBOS5-related genomic fragments.

Note that only the subset of the genomic restriction fragments homologous with pBOS5 which are specific for the *S*-locus genotype can be shown to segregate with the *S* alleles in the analysis reported here. Thus, these experiments do not completely rule out the possibility that the gene for SLSG is located on a segment of DNA unlinked to the *S* locus. If this is so, the SLSG polypeptide specified by this unlinked segment must be modified in some manner by a different molecular product of the *S* locus. However, the bands which exhibit polymorphisms are, in general, those which hybridize most strongly to the pBOS5 probe, and are thus most likely to have served as the template for the transcription of SLSG message.

At present, the simplest hypothesis consistent with the data asserts that SLSGs are in fact the products of the *S* locus. This assumption may be tested directly by a future demonstration that the transformation of plants with cloned genomic DNA selected with pBOS5 can alter their incompatibility specificity.

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LETTERS TO NATURE

A cosmic-ray explanation of the galactic ridge of cosmic X-rays

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Recent observations¹ with the Exosat X-ray satellite have confirmed the existence of an apparently diffuse galactic ridge of emission extending over the inner Galaxy ($l = 320^\circ$ – 40°) and confined to about $\pm 2^\circ$ in galactic latitude. On the basis of measurements² carried out in the galactic plane at $l \approx 55^\circ$, we have proposed previously³ that the diffuse galactic X-ray flux known at that time could be understood in terms of the synchrotron radiation of cosmic-ray electrons in the galactic magnetic field. The more extensive Exosat measurements fit nicely into this picture and the X-ray-producing electrons can now be readily understood as being secondary to the ultra-high-energy protons, which are themselves generated in galactic objects as epitomized by the recently discovered^{4,5} Cygnus X-3. We show here that, on this model, the number of Cyg X-3-like objects present in the Galaxy at any one time, averaged over the past 10^3 yr or so, is ≈ 30 .

The first report of an apparently diffuse X-ray flux from the galactic plane seems to have been by Cooke *et al.*⁶ in 1969. The most recent¹ work, using Exosat for two-dimensional mapping, strongly supports the existence of the ridge, and Warwick *et al.*¹ estimate an overall galactic diffuse emission of $\approx 1 \times 10^{38}$ erg s⁻¹ in the energy range 2–6 keV.

Figure 1 reproduces some of the Exosat results, together with a profile of the synchrotron emission at 408 MHz from ref. 7. The bulk of the 408-MHz radiation is due to GeV cosmic-ray electrons interacting with the galactic magnetic field. The similarity in the X-ray emission distribution with the synchrotron radiation profile is encouraging for our model but not conclusive, of course, because of the significant differences in spatial distributions of the X-ray-producing electrons ($\sim 10^{13}$ – 10^{15} eV) and the GeV electrons responsible for the 408-MHz radiation; the z (height above the galactic plane) distributions are very different.

The normalization in Fig. 1 is made at the minima in the observed X-ray profile in the region of $l \approx 30^\circ$ and 330° , because these low values should more closely represent the genuinely diffuse X-ray component between the sources (we consider that the minima at $|l| < 30^\circ$ are contaminated by contributions from unresolved discrete sources). The corresponding total X-ray emission is lower by a factor of ~ 2 than the estimate made by Warwick *et al.*¹ and is thus $\sim 5 \times 10^{37}$ erg s⁻¹. Another difference between our approach and that of Warwick *et al.*¹ is that we believe that the 'dips' in the X-ray profile, rather than reflecting

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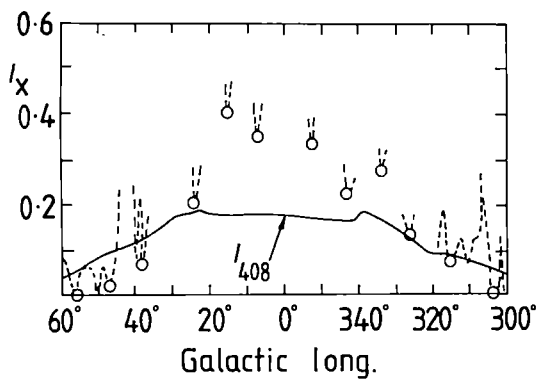


Fig. 1 Longitude distribution of the 'diffuse' X-ray emission, in the range 2–6 keV, from ref. 1 (Exosat data); the dashed line represents the profile and the circles the minima. The units are $7 \times 10^{-11} \text{ erg s}^{-1} \text{ cm}^{-2}$ per beamwidth. Also shown is the 'model' that we adopted based on the 408-MHz radio profile at $b = 0^\circ$ (ref. 7), normalized to the X-ray flux minima at $l \sim 30^\circ$ and 330° . The patchiness in the observed X-ray emission probably comes from both the presence of unresolved discrete sources, which are difficult to allow for, and the fact that the ultra-high-energy electrons, producing the synchrotron X rays, have only a limited diffusion away from their sources during the average electron lifetime of $\approx 2 \times 10^3 \text{ yr}$ against radiative losses.

excessive absorption, correspond to regions of lower intrinsic emission as well as inter-source regions.

The magnitude of the absolute electron flux that is compatible with the observed X-ray emission is of the order of $\sim 10^{-24} \text{ cm}^{-2} \text{ s}^{-1} \text{ sr}^{-1} \text{ eV}^{-1}$ at 10^{14} eV , as inferred by Protheroe and Wolfendale⁸ to explain the then known diffuse X-ray flux^{2,3}. This electron flux corresponds to an injection spectrum having a roughly constant exponent from much lower energies and it is higher than that based on an extrapolation of the local electron spectrum, known only up to particle energies of $\sim 10^{12} \text{ eV}$. The argument advanced then⁸ to account for this apparent disparity was that we are not near enough to an ultra-high-energy electron source, at present, to measure the actual average galactic intensity, which was admittedly a rather *ad hoc* justification.

The new development that has strengthened the above argument, in particular for a significant contribution to the galactic X-ray ridge coming from the cosmic ray electrons, has been the discovery^{4,5} of ultra-high-energy γ rays, up to 10^{16} eV , from Cyg X-3, a binary system about 12 kpc from the sun. The γ rays are probably^{9,10} secondary to the protons which are first accelerated in the electromagnetic fields of one of the binary components (neutron star?) and then interact with the gas present in the atmosphere of the companion star. As a result, there will be electrons associated with the $\pi^\pm \rightarrow \mu^\pm \rightarrow e^\pm$ cascades corresponding to γ rays from the alternative π^0 -decay chain. The ratio of electrons to γ rays cannot be calculated accurately because of insufficient knowledge about the production region, but it is unlikely to be far from the factor 0.5 expected on the basis of kinematics, and we adopt this value here. We assume that the electrons can escape from the 'source region' and are not trapped by magnetic fields, a reasonable assumption in that the parent protons must have had near-rectilinear motion to preserve the γ -ray phase.

Concerning the energetics of the process, $\gamma \rightarrow e \rightarrow \text{X rays}$, we note that the γ -ray energy per decade from Cyg X-3 (1978 level⁴) is $\sim 10^{37} \text{ erg s}^{-1}$, centred on $5 \times 10^{14} \text{ eV}$, the energy required to give X rays in the middle of the detected range (2–6 keV). The X-ray energy is proportional to the square of the electron energy so that the progenitor-electrons will have energy $E_e \sim (4\text{--}7) \times 10^{14} \text{ eV}$, corresponding to the Cyg X-3 output of $0.5 \times 3 \times 10^{36} \text{ erg s}^{-1}$, that is, $1.5 \times 10^{36} \text{ erg s}^{-1}$.

If, as is likely, Cyg X-3-type sources represent a typical galactic population, such as pulsars, supernova remnants and

H II regions, and have a spatial distribution similar to that of the GeV cosmic-ray electrons¹¹ responsible for the radio synchrotron emission—a peak intensity of about twice the local value at a galactocentric distance $R = 6 \text{ kpc}$ —then the longitude distribution will be similar to that denoted by I_{408} in Fig. 1.

As was pointed out earlier, the total energy requirement in electrons is $\sim 5 \times 10^{37} \text{ erg s}^{-1}$ to account for the Exosat X-ray ridge so that there should be ~ 30 sources contributing, each emitting $\sim 1.5 \times 10^{36} \text{ erg s}^{-1}$ for $E_e \sim (4\text{--}7) \times 10^{14} \text{ eV}$. Note that this is not necessarily the number 'switched on' at present because the mean lifetime of the electrons due to the synchrotron losses in the galactic magnetic field is $\sim 2 \times 10^3 \text{ yr}$ at $E_e \sim 5 \times 10^{14} \text{ eV}$. Thus, this number corresponds to the average value over a time of this order.

The number appears to be large but is not implausible, as is evident from the following observations. (1) There is a suggestion¹² that Cyg X-3 has been considerably stronger in the past and the ultra-high-energy photon flux ($\sim 10^{15} \text{ eV}$) may have decreased by a factor as large as ~ 80 in the past decade only¹³, for which the data are available. (2) A source (LMC X-4), stronger than Cyg X-3 by a factor ~ 20 , has been reported¹⁴ in the Large Magellanic Cloud. (3) Cosmic-ray anisotropy measurements at $> 10^{15} \text{ eV}$ suggest that Cyg X-3 is not unique and that there may be up to 10 similar sources in the Galaxy at present¹⁵. Similarly, at lower energies, between 1 and $3 \times 10^{13} \text{ eV}$, the recent anisotropy measurements¹⁶ at Baksan Valley and Norikura indicate¹⁷ an 'excess' near Right Ascension (RA) $\sim 300^\circ$ with respect to the general flow of particles from RA: $40^\circ\text{--}100^\circ$. If interpreted as due to γ rays, this excess leads to an upper bound of ~ 70 on the number of sources operating in the Galaxy at present (see ref. 17). (4) A recent analysis of muon-poor showers by the Lodz group¹⁸ indicates an excess of 1–2% from the galactic plane at $\sim 2 \times 10^{16} \text{ eV}$, a result which is consistent with the presence of 10–15 Cyg X-3-like sources in the Galaxy at the present epoch¹⁷.

Finally, we consider the latitude dependence of the X-ray flux due to the cosmic-ray electrons. The short lifetime of these electrons ($\sim 2 \times 10^3 \text{ yr}$) means that they will rarely travel beyond 100 pc from their production points. Now the z distribution of the sources is probably of a similar magnitude (young stars have a scale-height $z_1 \sim 70 \text{ pc}$; (ref. 19), so that the z distribution of the energetic electrons is unlikely to be wider than that derived for the X-ray emissivity by Warwick *et al.*¹ from the experimental data.

All we now need is a test for the present hypothesis. The electrons, produced by the decay of charged pions, will have a differential exponent γ_d of 1.8 (for Cyg X-3 γ rays) to 2.8 depending on whether the target material, where the pions are produced, is thin or moderately thick. This exponent is likely to hold for electron energies of up to a few times 10^{15} eV , after which it may steepen sharply, as suggested by the spectral cutoff seen⁵ in the Cyg X-3 γ rays. As the synchrotron X rays ($< 10 \text{ keV}$) are produced by electrons of energy $10^{14}\text{--}10^{15} \text{ eV}$, we expect them to have a power-law spectrum with $\gamma_d = 1.4\text{--}1.9$. The observed spectrum may actually be steeper by $\Delta\gamma_d \approx 0.5$ because of the shorter lifetime of the higher energy electrons. The Exosat spectral data¹ seem to be consistent with thermal bremsstrahlung ($kT \sim 4\text{--}20 \text{ keV}$) or with a power law ($\gamma_d = 1.1\text{--}4.6$), and our prediction is in the middle of this range. It is to be hoped that better-quality spectral measurements will soon become available on the diffuse X-ray component and will further constrain the model spectrum.

The observation of the exponent as predicted here would go a long way towards establishing the phenomenon or, if the observed spectrum were steeper, in enabling an upper limit to be placed on the cosmic-ray electron contribution. In a sense, this test is complementary to the prediction by Rana *et al.*¹² of the presence of hard X rays, in the region of 100 keV, at high galactic latitudes. These X rays are likely to be due to synchrotron radiation by electrons, which are themselves produced in the halo following collisions of ultra-high-energy γ rays from Cyg X-3-like sources with the 3K radiation photons.

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An active current sheet in the solar wind

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The supersonic solar wind possesses a variety of simple small scale (<10-min observation duration) phenomena, including discontinuities^{1–3} and magnetic holes^{4,5}. We report here recent AMPTE-UKS⁶ (Active Magnetospheric Particle Tracer Explorer–UK Satellite) observations, however, which reveal a dramatic new complex structure within the solar wind flow. In the plasma and field observations of this magnetic hole/current sheet system, the layers surrounding the strong rotation in the magnetic field contain compressed and heated solar wind, an enhanced field strength and considerable wave activity. The interior region shows strong ion heating but with ambient densities and a fluctuating magnetic field which dips to low values. The bulk flow velocity inside is deflected sharply from the anti-sunward direction, but not in the direction predicted by magnetic stresses across the boundary. The structure is expanding in thickness and is relatively young. The source of momentum and energy for this event remains unclear, and could even be magnetospheric.

Most structures observed by *in situ* satellite measurements in the collisionless solar wind plasma have relatively simple, monotonic profiles and are either passive, convecting entities or shocks. The present observations seen by the AMPTE-UKS reveal an apparently more active current sheet. An important aspect of this work is the combination of high-time resolution three-dimensional particle data and wave data with the traditional magnetic field analyses on which earlier studies have been based. Recent, independent work⁷ using the ISEE spacecraft reports eight events which are very similar to the one presented here and provides a complementary range of data analysis.

Figure 1 displays five minutes of field and bulk plasma data taken in the solar wind on 30 October 1984. The spacecraft was

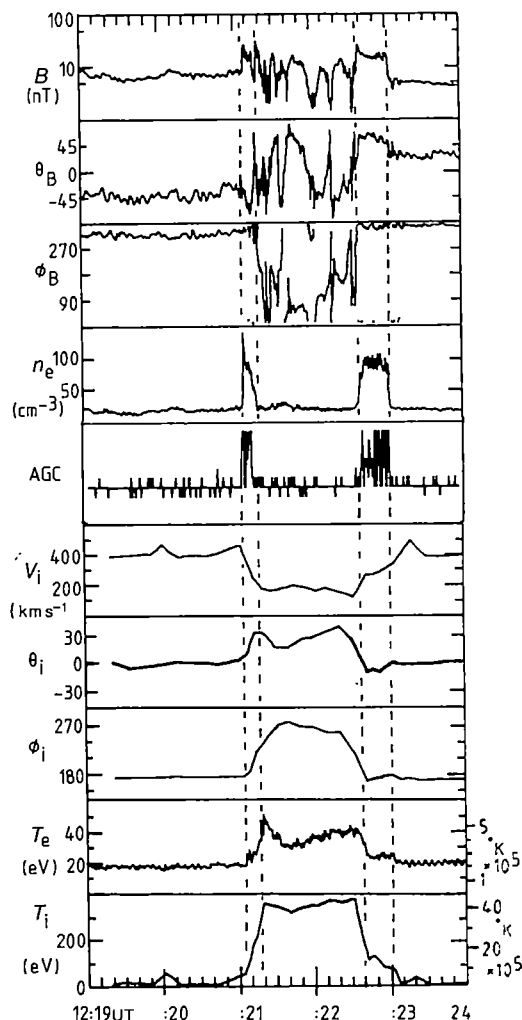


Fig. 1 Solar wind observations taken on 30 October 1984 by the AMPTE-UKS. Panels give (top to bottom) the magnetic field strength, latitude and azimuthal direction in geocentric solar ecliptic (GSE) coordinates (the sunward direction is $\theta = 0$, $\phi = 0$ with $\theta = 90$ being northward and $\phi = 270$ downward), electron number density, wave autocorrelator automatic gain control (AGC) level, ion flow velocity, flow latitudinal and azimuthal directions, and electron and ion temperatures. The electron measurements are based on individual spectra and could be averaged over one spin period (~ 5 s) to obtain the mean values. The ion parameters are taken on alternate spins when the ion instrument resolved either the solar wind component or uniform 4π coverage. The ion density, not shown, mimics n_e . The wave AGC level responds in successive factors of 2 of wave electric field amplitude in the 0.11–3.9 kHz band.

located in the dawn sector (approximately 09:30 LT) at a position relative to the Earth's centre of $11.3 R_E$ sunward, $9.7 R_E$ downward and $0.22 R_E$ northward out of the solar ecliptic plane, placing it some $1.2 R_E$ upstream of the point where the satellite crossed the Earth's bow shock 80 min earlier. The magnetic field data are 0.5-s averages. The electron data are based on individual spectra covering the range 12 eV–16 keV taken at ≈ 1 -s intervals, with moments computed on the assumption of an isotropic distribution. Departures from isotropy thus appear as 5-s spin-modulated moments. The ion data shown have a time-resolution of ≈ 10 s.

On a longer timescale, the solar wind preceding the event, and until the penultimate dashed vertical line in Fig. 1, was disturbed by the presence of backstreaming ion beams and/or diffuse ions, indicating a magnetic connection to the Earth's bow shock. The magnetic field showed large amplitude fluctuations, with $\delta|B| \approx 2$ nT, about a mean field of $\langle |B| \rangle \approx 7$ nT. The large southward to northward rotation in latitude of the

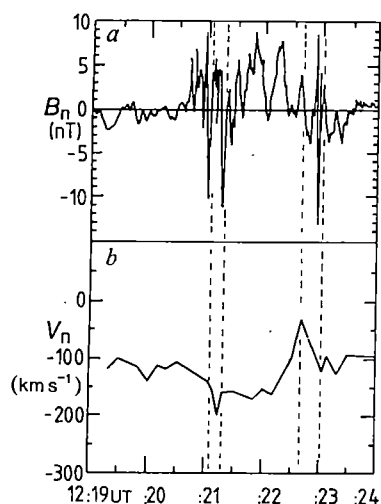


Fig. 2 Minimum variance component of the magnetic field (*a*) and corresponding ion velocity component (*b*). The normal direction is $\hat{n} \approx (0.38, 0.90, -0.22)$ in GSE (*x, y, z*) coordinates.

solar wind magnetic field from before to after the delineated event, seen in the second panel of Fig. 1, resulted in an exit from the ion foreshock, and a period of smooth solar wind with $\langle |B| \rangle \approx 5$ nT followed the interval shown here. The dashed vertical lines separate the obvious feature into three distinct regions.

The 'central' region is characterized by a varying magnetic field with several holes or depressions in field strength down to ≈ 1 nT. This is accompanied by hot electrons and even hotter ions, the latter dominating the total pressure here (see Table 1). The bulk flow shows a dramatic deceleration (from 400 to 150 km s⁻¹) and deflection (through $\approx 90^\circ$) from the ambient solar wind, resulting in a marked drop in bulk flow energy in the spacecraft frame of reference (see Table 1). Curiously the number density here returns to values comparable to those in the solar wind. The central region maintains rough pressure balance with the surrounding layers and is separated from them by a passive tangential discontinuity (the low central density eliminates the possibility of a shock transition).

'Edge' regions of enhanced field strength and density, accompanied by a partial deceleration, deflection and heating of the solar wind plasma but with little change in field direction,

Table 1 Pressures and energy densities (10^{-11} J m⁻³)

Parameter	Expression	Solar wind	Edge	Central
Magnetic pressure	$ B ^2/2\mu_0$	1.1	11	0.04–7.8
Ion thermal pressure	$n_i k T_i$	0.75	49	65
Electron thermal pressure	$n_e k T_e$	5.2	35	12
Normal bulk KE density	$\frac{1}{2} n_i 1.16 m_p V_n^2$	7.2	7.3	29
Transverse bulk KE density	$\frac{1}{2} n_i 1.16 m_p V - V_n \hat{n} ^2$	100	220	5.9

The ion densities used to compute this table were those actually measured and were a factor of 2–3 smaller than n_e due to differing experimental response/calibration.

* Assuming 4% helium by number.

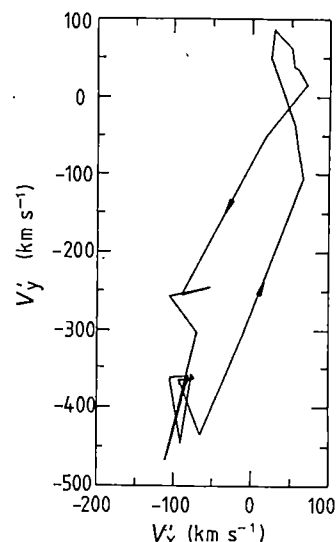


Fig. 3 Hodogram of the ion bulk velocity in the minimum variance plane of the magnetic field. The \hat{x}' direction corresponds to the maximum variance eigenvector, $\approx (0.21, 0.14, 0.97)$ GSE, while \hat{y}' is the intermediate eigenvector $\approx (0.90, -0.41, -0.14)$ GSE. Note that the velocity deflection is not aligned with the magnetic field maximum variance direction.

surround the central region. The automatic gain control (AGC) level of the wave experiment in this region gives r.m.s. wave amplitudes of the order of 1 mV m⁻¹ across this low-frequency band. Such strong fields usually indicate the presence of significant wave-particle interactions. Fast shocks probably form the outer boundaries of these regions although it is difficult to determine the downstream flow well enough to test this with any confidence. The particle correlator experiment on the satellite had sufficient time during the later trailing edge to reveal modulations over a range of frequencies (0–3 kHz) in the count rate of ≈ 100 eV electrons. This similarity to the response seen at the Earth's (fast) bow shock supports our supposition of a shock at the outer boundary. Table 1 shows that the total (plasma plus field) pressure in these edge regions is comparable to that of the central section, and that both considerably exceed the exterior pressure.

A minimum variance analysis of the field yields a well-defined normal direction (intermediate/minimum eigenvalue ≈ 4), \hat{n} , although Fig. 2 reveals quite strong fluctuations in the normal component of B . Figure 2 also shows the component of the ion bulk velocity along this normal. The average value of ~ 125 km s⁻¹ yields an estimated thickness of 15,000 km for the entire 2-min event. There is also a suggestion that the magnitude of V_n increases on entrance and decreases on exit, implying an expansion of the region by ± 50 –75 km s⁻¹. This interpretation is dependent on the normal direction which, given B_n from Fig. 2, may be inaccurate. Nonetheless, combining the expansion speed and thickness yields a maximum age of ~ 2 min for this active phase of the current sheet.

Figure 3 completes our analysis by displaying the ion bulk velocity in the plane of the current sheet. The large deflection of ~ 500 km s⁻¹ occurs primarily along the (\hat{y}') intermediate variance direction. Tangential stress balance, however, (if $B_n \neq 0$) predicts that ΔV should be along the maximum variance direction \hat{x}' (parallel to $\pm \Delta B$). Indeed, Fig. 2 has already indicated an average B_n which is consistent with zero. This central flow cannot correspond to the simple magnetic reconnection picture⁸ nor to that expected in smaller scale flux transfer events⁹ and must be bounded by purely tangential discontinuities. We conclude that the flow is driven predominantly by a strong pressure gradient within the sheet itself. Figure 4 summarizes the various field and flow directions deduced above.

The momentum and energy source responsible for the remarkable deflection and heating of the solar wind seen in this event

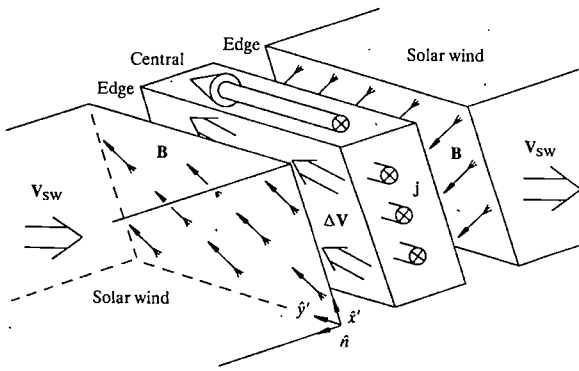


Fig. 4 Sketch of the geometry inferred from the data for the current sheet system, including the $(\hat{x}', \hat{y}', \hat{n})$ variance coordinate system of the magnetic field, \mathbf{B} (thin arrows), along with the current density, \mathbf{j} (cylindrical arrows), and bulk flow direction of the solar wind, \mathbf{V}_{sw} , and its deflection, ΔV , within the sheet (double arrows).

is not clear. This pressure pulse could have been generated by a fortuitously recent burst of magnetic reconnection located within the current sheet but some distance away from the satellite. On the other hand, we cannot eliminate the possibility of magnetospheric influence, most probably at the bow shock or within the magnetosheath. Tracing backwards along the velocity vector in the central region we reach a point of intersection with the bow shock (based on a fit to the bow shock¹⁰ normalized to its crossing 80 min earlier the same day). The time required to traverse this distance ($\sim 3.75 R_E$) is longer than our estimated maximal age, but only by 7%, which is well within our uncertainties in age, direction and bow shock position. Thomsen *et al.*⁷ discuss thoroughly (and cast doubt on) a variety of possible source hypotheses for their observed events.

Our data show a solar wind current sheet containing a strong, young pressure pulse which forces it to expand and drives a flow within the sheet itself. The hot, deflected interior is dominated by ion thermal pressure, and is separated from the ambient solar wind by regions of shocked or compressed solar wind. We have no explanation of the near solar wind densities inside the sheet, although initial compression/heating followed by the sheet's expansion may have conspired to provide this as a coincidence. We have not succeeded in identifying the energy source or triggering mechanism, which could be either magnetospheric or interplanetary in origin.

We have seen other examples in the AMPTE-UKS data set which display similar features to those presented here. A previously reported solar wind event¹¹ also showed some of the same characteristics.

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A gravitational analogue of Faraday rotation

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In an effort to shed some light on the problem of nonlinear interaction of gravitational radiation we have studied the behaviour of cylindrical gravitational waves in vacuum. When only one mode of cylindrical gravitational waves is present, the well known solution is the Einstein-Rosen¹ waves which do not display any nonlinear behaviour. The general solution of two modes is unknown. To study it we express the cylindrical Einstein equations in a characteristic form and obtain an analytical approximation and a numerical solution for the general evolution of cylindrical waves. We report here that the propagation of these waves displays a reflection of ingoing to outgoing waves (and vice versa), combined with a rotation of the polarization vector between the + and × modes. The latter effect is a gravitational analogue of the electromagnetic Faraday rotation.

The general cylindrical Kompaneets Jordan Ehlers line element^{2,3}

$$ds^2 = e^{2(\gamma-\psi)}(dt^2 - dr^2) - e^{2\psi}(dz + \omega d\theta)^2 - r^2 e^{-2\psi} d\theta^2 \quad (1)$$

involves three functions ψ , ω , and γ , of r and t only. ψ and ω represent the two dynamical degrees of freedom of the gravitational field, + and × respectively; while γ plays the role of the total gravitational energy⁴. The Einstein equations in vacuum

$$\psi_{,tt} - \frac{\psi_{,r}}{r} - \psi_{,rr} = (e^{4\psi}/2r^2)(\omega_{,t}^2 - \omega_{,r}^2) \quad (2)$$

$$\omega_{,tt} + \frac{\omega_{,r}}{r} - \omega_{,rr} = 4(\omega_{,r}\psi_{,t} - \omega_{,t}\psi_{,r}) \quad (3)$$

$$\gamma_{,r} = r(\psi_{,t}^2 + \psi_{,r}^2) + \left(\frac{e^{4\psi}}{4r}\right)(\omega_{,t}^2 + \omega_{,r}^2) \quad (4)$$

$$\gamma_{,t} = 2r\psi_{,r}\psi_{,t} + \left(\frac{e^{4\psi}}{2r}\right)\omega_{,t}\omega_{,r} \quad (5)$$

can be cast into a simpler characteristic form. To do so we define the ingoing and outgoing coordinates $u = \frac{1}{2}(t-r)$, $v = \frac{1}{2}(t+r)$; and the ingoing, I_+ , I_\times and outgoing, O_+ , O_\times amplitudes

$$I_+ = 2(\psi_{,t} + \psi_{,r}) \quad (6)$$

$$O_+ = 2(\psi_{,t} - \psi_{,r}) \quad (7)$$

$$I_\times = \frac{e^{2\psi}}{r}(\omega_{,t} + \omega_{,r}) \quad (8)$$

$$O_\times = \frac{e^{2\psi}}{r}(\omega_{,t} - \omega_{,r}) \quad (9)$$

The indices + and × denote the respective polarizations. With these definitions equations (2)-(3) become a set of four, first-order, coupled equations (R. F. Stark, personal communication):

$$I_{+,u} = \frac{I_+ - O_+}{2r} + I_\times O_\times \quad (10)$$

$$O_{+,v} = \frac{I_+ - O_+}{2r} + I_\times O_\times \quad (11)$$

$$I_{\times,u} = \frac{I_\times + O_\times}{2r} - I_+ O_\times \quad (12)$$

$$O_{\times,v} = -\left(\frac{I_\times + O_\times}{2r}\right) - O_+ I_\times \quad (13)$$

These equations are supplemented with the boundary conditions

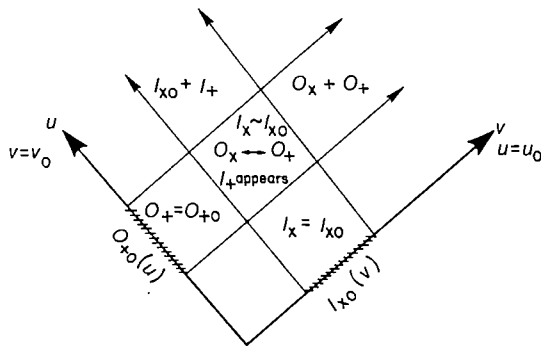


Fig. 1 Evolution for large r . The initial data are O_{+0} and I_{x0} . The initial waves propagate freely, until they cross each other. In the interaction region, O_+ and O_x rotate and a small amount of I_{x0} is converted to I_+ (see equations (17)–(20)). When leaving the interaction region, the rotation stops, and the ingoing and outgoing waves include both polarizations.

at the origin

$$I_+ = O_+ \quad \text{and} \quad I_x = -O_x \quad \text{at } r=0 \quad (14)$$

The initial conditions for equations (10)–(13) can be given either on a space-like hypersurface or on two null hypersurfaces, the latter being the simplest.

The first term on the right-hand side of equations (10)–(13), couples the ingoing and outgoing waves with the same polarization, giving rise to the usual cylindrical reflection. At $r=0$, this reflection is complete, and the ingoing waves turn into outgoing ones.

The other term describes a nonlinear interaction between the two polarizations. To investigate the nature of this interaction, we consider a large enough r , so that we can neglect the first terms in the right-hand side of equations (10)–(13). In this approximation, the solution of equations (10)–(13) becomes

$$I_x(u, v) + iI_+(u, v) = (I_{x0}(v) + iI_{+0}(v)) \exp \left[i \int_{u_0}^u O_x(u', v) du' \right] \quad (15)$$

$$O_x(u, v) + iO_+(u, v) = (O_{x0}(u) + iO_{+0}(u)) \exp \left[i \int_{v_0}^v I_x(u, v') dv' \right] \quad (16)$$

$I_{+0}(v)$, $I_{x0}(v)$, $O_{+0}(u)$ and $O_{x0}(u)$ are the initial conditions given on an outgoing ($u=u_0$) and an ingoing ($v=v_0$) null hypersurface respectively. When I_x is present, O_+ and O_x will oscillate according to equation (16) with a phase difference of $\pi/2$. This means that if the initial outgoing wave is linearly polarized its polarization vector will rotate as it propagates through the $I_x \neq 0$ region. The same is also true for the incoming waves (equation (15)).

For example, consider the case where $O_{x0} = I_{+0} = 0$ and $|O_{+0}| \ll |I_{x0}|$. I_x almost does not change:

$$I_x(u, v) \approx I_{x0}(v) \quad (17)$$

O_+ and O_x oscillate when the O_+ wave crosses the $I_x \neq 0$ region

$$O_+(u, v) \approx O_{+0} \cos \left(\int_{v_0}^v I_{x0}(v') dv' \right) \quad (18)$$

and

$$O_x(u, v) \approx -O_{+0} \sin \left(\int_{v_0}^v I_{x0}(v') dv' \right) \quad (19)$$

The appearance of O_x causes a conversion of a small part of I_x to I_+ and if $|O_{+0}| \ll 1$ we have

$$I_+(u, v) \approx -I_{x0} \int_{u_0}^u O_{+0} du' \sin \int_{v_0}^v I_{x0}(v') dv' \quad (20)$$

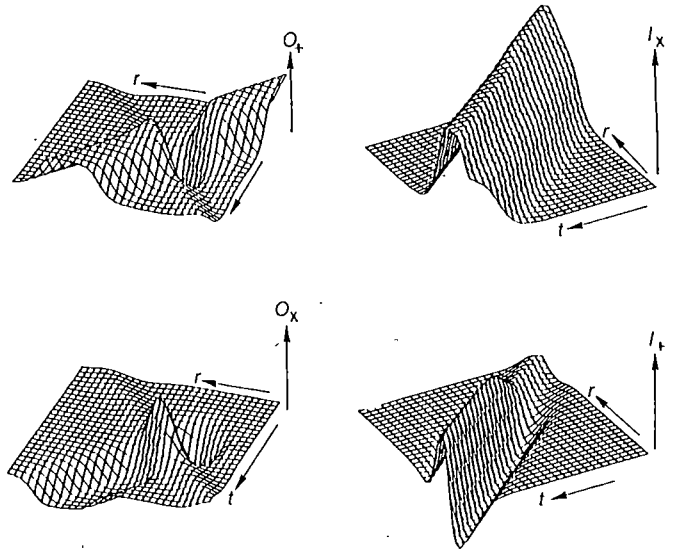


Fig. 2 I_x , I_+ , O_x and O_+ obtained by numerical solution⁶ of the exact equations (10)–(13). The initial data are $I_{+0} = O_{x0} = 0$, $|I_{x0}| \gg |O_{+0}|$, $|O_{+0}| \ll 1$. Note that the vertical scale is not the same in the different pictures. The behaviour matches the approximated equations (17)–(20) very well.

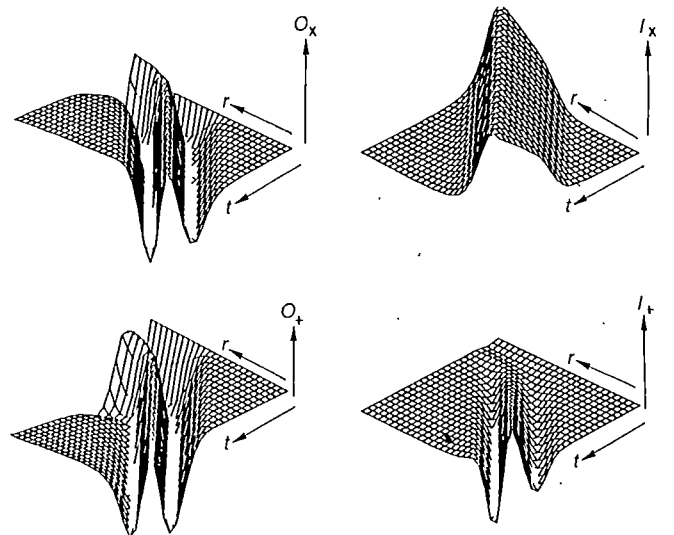


Fig. 3 Numerical results for initial data containing only an ingoing, x polarized pulse. Radiation in the I_+ , O_x and O_+ waves appears, and these waves rotate. Note that O_x and O_+ are out of phase by $\pi/2$. After O_x and O_+ leave the region where I_x is present the rotation stops (the vertical scaling is different for each section).

This behaviour is displayed in Figs 1 and 2. After the waves cross each other the rotation stops and the polarization vector freezes.

In general, when both O_{x0} and I_{x0} are large, each wave will cause a rotation of the polarization vector of the other.

The rotation of the polarization vector is due solely to the nonlinear nature of the gravitational interaction, that is, to the terms on the right-hand side of equations (2), (3). In electromagnetism there is no such interaction. However, in the presence of a magnetic field and plasma, the polarization vector of an incident linearly polarized wave rotates—a phenomenon known as Faraday rotation. Here the ingoing waves (say), play the role of both the plasma and the magnetic field as they rotate the polarization vector of the outgoing waves propagating through them.

One has to worry now about the combined effect of all terms in equations (10)–(13). If the initial data contains only the $+$ mode the sole effect will be the cylindrical reflection of I_+ to O_+ . These are the Einstein–Rosen waves. More complicated

behaviour emerges when the \times mode is present in the initial data. We observe both conversion of I_+ to O_+ and I_\times to O_\times (via the first term in equations (10)–(13)) and rotation between I_+ and I_\times and O_+ and O_\times taking place simultaneously.

For example, an initial I_\times pulse will reflect some O_\times wave. This O_\times wave will, in turn, cause a rotation of some of the initial I_\times pulse into an I_+ pulse and by itself will rotate (due to the presence of I_\times) into O_+ (see Fig. 3).

Does this simple analysis overlook some features of the interaction of cylindrical gravitation waves, such as formation of singularities? To answer this question, with R. F. Stark, we have solved numerically equations (10)–(13). The exact solution (Figs 2, 3) demonstrate the validity of this simple picture and its completeness. A detailed description of these results will be given elsewhere⁵.

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Helium isotopes in North Sea gas fields and the Rhine rift

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The popular assumption that all natural hydrocarbon accumulations are biogenic in origin has recently been questioned^{1,2} with the suggestion that deep source (mantle-derived) hydrocarbons may contribute to accumulated biogenically produced hydrocarbons, and also give rise to hydrocarbon deposits in unexpected parts of the crust. The possibility that deep-source volatiles are present in significant quantities in the hydrocarbon-bearing parts of the crust of western Europe is assessed here using analyses of ³He abundances in groundwaters and natural gas accumulations within the UK mainland and continental shelf. For comparative purposes, analyses are also presented for groundwaters from the southern part of the Rhine Graben, a region that has been relatively active tectonically, and the site of Tertiary and Quaternary volcanic activity. In contrast, the North Sea sedimentary basins and Central Graben have no recorded Tertiary or younger volcanic activity. Helium associated with North Sea gas fields and groundwaters on the UK mainland is dominantly radiogenic, produced by decay of U and Th in the continental crust, even where heat flow exceeds 100 mW m⁻². A minor component (<1%) of mantle-derived helium with primordial ³He, may be present in some gas fields close to the North Sea Central Graben. Further south along the more active part of the same fault system in the Rhine Graben this component reaches >15% in groundwater samples. Fault structures seem to be important for the transport of mantle helium through the brittle upper crust. The results are entirely consistent with a biogenic origin for North Sea hydrocarbons.

Table 1 and Fig. 1 show ³He/⁴He and He/Ne ratios for 10 natural gas samples from the UK shelf and mainland; analyses were made as described elsewhere³. Nine of the samples are from the North Sea basins including the Ninian, Frigg, Forties fields in the north, and Placid, Indefatigable, Viking, Doty, Hewett and Leman in the south, and one sample is from the Wytch Farm field in southern England. He/Ne ratios are high,

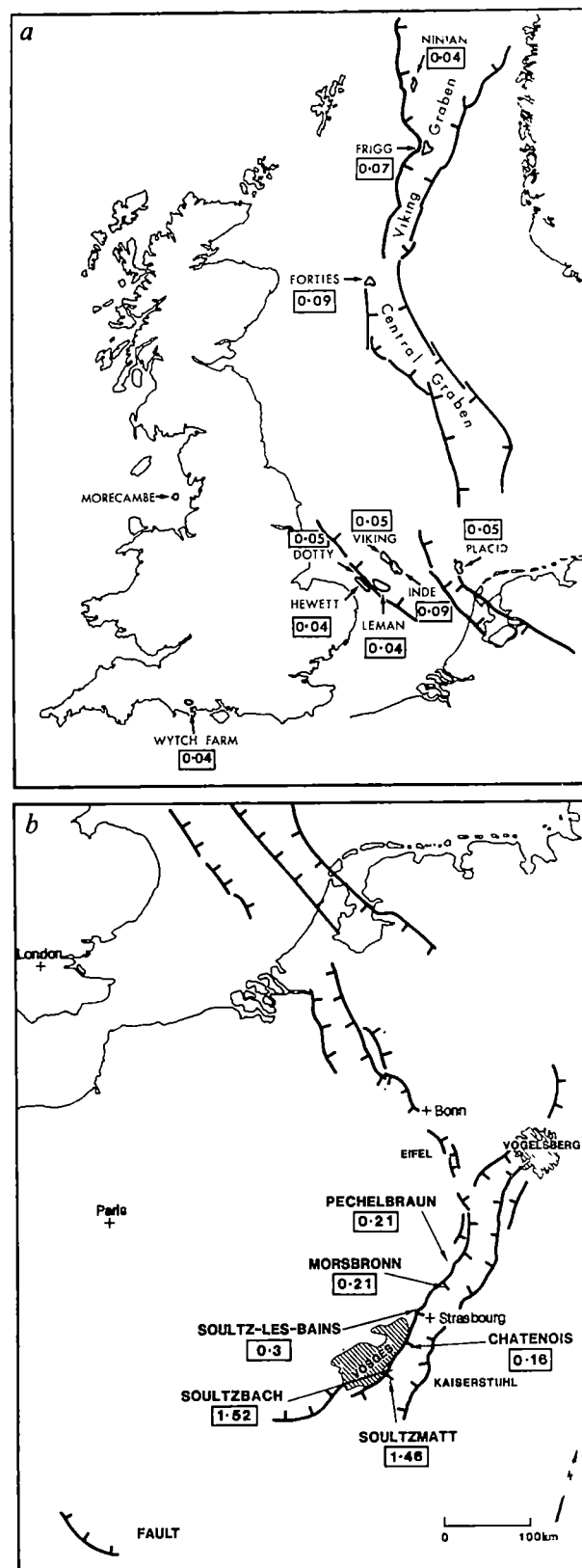


Fig. 1 a, Location of gas fields sampled for helium isotope analyses. b, Location of groundwater samples from the southern Rhine Graben. Numbers shown on maps are R/R_a values for samples analysed.

mostly >100, and indicate that any atmosphere-derived rare gas component is negligible in all cases except the Wytch Farm sample. Corrected $(R/R_a)_c$ ratios from the natural gas samples have a restricted range between 0.036 and 0.091, and where duplicate analyses have been made (Viking, Doty, Indefatigable, Frigg and Ninian) the ratios agree within several per cent. (R/R_a) is the ratio of the sample ³He/⁴He to that of the

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Table 1 Helium results for natural hydrocarbon gas samples from the North Sea area and for groundwater samples from the Alsace region

Sample	R/R_a	He/Ne	$(R/R_a)_c$	[He]	Sample	R/R_a	He/Ne	$(R/R_a)_c$	[He]
(10 ⁻⁹ nl per g H ₂ O)					(10 ⁻⁹ nl per g H ₂ O)				
Hydrocarbons					Groundwaters				
G1473	0.045	350	0.044		Soultzmatt	1.35	100	1.35	19.5
Hewett					No. 2				
G1473 repeat	0.046	182	0.045		Soultzmatt	1.47	264	1.47	12.1
G1973	0.054	75	0.051		No. 3				
Viking					Soultzmatt	1.46	111	1.46	23
G1973 repeat	0.054	139	0.052		No. 4				
G2226	0.042	175	0.040		Soultzbach	1.53	168	1.53	13
Leman					No. 1				
G2147	0.047	142	0.045		Soultzbach	1.51	75	1.51	8.8
Dotty					No. 2				
G2147 repeat	0.048	122	0.046		Morsbronn	0.216	359	0.216	0.12
G2640	0.049	113	0.047		Arbogast No. 1				
Placid					Morsbronn	0.212	512	0.211	382
G2665	0.095	59	0.091		Curraier				
Indefatigable					Pechelbraun	0.212	480	0.212	263
G2665	0.089	305	0.089		71 °C				
new aliquot					Chatenois	0.153	64	0.150	101
G2390	0.099	10	0.072		Chatenois	0.159	311	0.159	439
Frigg					No. 2				
G2390	0.069	421	0.068		Soultz les Bains	0.301	177	0.300	1,230
new aliquot					Dept 67, No. 1				
G2706	0.037	302	0.036		Soultz les Bains	0.296	372	0.296	297
Ninian Field					Dept 67, No. 2				
(associated gas)									
G2706 repeat	0.036	495	0.036						
G2186	0.092	59	0.087						
Forties Field									
(associated gas)									
G2359	0.172	2.1	0.036						
Wytch Farm									
(associated gas)									

R/R_a is the ratio of the sample $^3\text{He}/^4\text{He}$ to that of air (1.4×10^{-6}). The sample He/Ne ratios ($\pm 10\%$) were used to correct for air-derived helium in the sample by the expression $(R/R_a)_c = [(R/R_a)X - 1]/(X - 1)$ where X is $(\text{He/Ne})_{\text{sample}}/(\text{He/Ne})_{\text{air or water } 10^\circ\text{C}}$. For the gas samples $(\text{He/Ne})_{\text{air}} = 0.288$ was used, and for the groundwaters $(\text{He/Ne})_{\text{water}} = 0.230$. Neon is regarded as being purely of atmospheric origin. Generally, errors in R/R_a are $\pm 3\%$ at the 95% level of confidence, in agreement with the comparisons afforded by the duplicates carried out on several of the hydrocarbon samples. The [He] values for the groundwaters have uncertainties of $\pm 10\%$.

atmosphere.) $^3\text{He}/^4\text{He}$ ratios of crustal helium sampled by groundwaters are similarly low. Samples from the Lincolnshire limestone aquifer in eastern England, the Marchwood borehole, Hampshire, and return waters from the Carnmenellis granite, artificially fractured as part of a Hot Dry Rock Project, have R/R_a values between 0.014 and 0.04 (Table 1, Fig. 2). The highest R/R_a values currently known from the UK mainland and shelf are from gas fields intimately associated with boundary faults to the Central Graben structure in the North Sea.

$^3\text{He}/^4\text{He}$ and He/Ne ratios are presented in Table 1 for 12 groundwaters from Alsace, several of which are currently exploited as mineral water sources. All samples have high He/Ne ratios, mostly > 100 , and have insignificant atmosphere-derived helium components, with higher and more variable R/R_a values ($0.14 < R/R_a < 1.5$) than those found on UK mainland and shelf. All of the Alsace samples show higher levels of ^3He -enrichment than any of the UK samples.

The presence of a small amount of mantle-derived helium in the continents should be recognizable even when diluted by very much larger amounts of radiogenic helium (Fig. 2). However, the estimation of its abundance depends critically on the precise R/R_a values assigned to the mantle and crustal components. The composition of the present-day mantle-derived component, typified by the release at spreading ridge axes, is well known, and more uncertainty surrounds the appropriate values for the radiogenic production ratio of helium in the crust. Early estimates of the production ratio⁴, which assumed the ^6Li (n, α) ^3H reaction to be the dominant ^3He -producing reaction, suggested R/R_a values for crustal helium of 0.1. More recent estimates⁵ of neutron-producing reactions in crustal rocks suggest a production value of $R/R_a = 0.02$, for typical continental

crust. In several situations, good agreement has been reported between the measured R/R_a values of helium and the predicted values for particular lithologies⁵. The above deductions are certainly consistent with the results reported for the groundwaters and for at least some of the hydrocarbon reservoirs in the UK, which contain helium derived from large rock volumes. Some departure from these 'typical' values is to be expected on the scale of individual minerals or fluid inclusions where large variations in Li abundance and in effective neutron flux, will occur; thus R/R_a values > 0.1 have been reported for particularly Li-rich minerals. Such high production ratios will not, however, be characteristic of large segments of continental crust, and the high values reported from Alsace are interpreted in terms of an admixture of mantle helium with crustal radiogenic helium. A few of the North Sea gas fields, particularly Forties and Indefatigable, have R/R_a values significantly greater than that predicted for crustal radiogenic helium, implying either a small component of mantle-derived helium mixed with 'normal' crustal helium, or the presence of crustal helium with an abnormal R/R_a value in the natural gas reservoir. The latter possibility is difficult to assess, but would presumably require that locally Mesozoic sediments have abnormally high Li-contents and that these are degassing preferentially, or alternatively Li-rich mineral components lose their helium selectively. In the absence of appropriate chemical information on the sediments themselves, this possibility cannot be excluded, but the close proximity and control of North Sea gas fields by fault structures, coupled with the observed large excesses in ^3He associated with similar structures in the southern Rhine Graben, suggests that a trace ($\sim 1\%$) component of mantle helium has entered the Forties and Indefatigable fields.

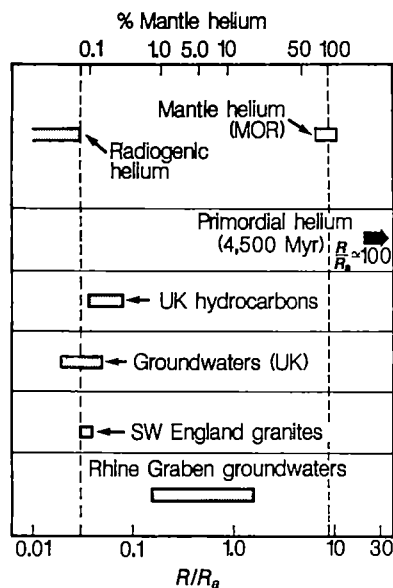


Fig. 2 The isotope composition of helium in samples from western Europe compared with radiogenic and mantle helium from mid-ocean ridges (MOR). $^3\text{He}/^4\text{He}$ ratios of samples (R) are compared with the $^3\text{He}/^4\text{He}$ ratio of the atmosphere, $R_a (=1.4 \times 10^{-6})$. The observed variations are interpreted as mixtures of radiogenic helium ($R/R_a < 0.03$) and mantle-derived helium possessing a primordial ^3He component with $R/R_a = 9$. The corresponding percentage of mantle helium on this basis is indicated at the top of the figure. The estimated composition of radiogenic helium is taken from ref. 5. Results for the Rhine Graben and UK hydrocarbons are from Table 1 and those for UK groundwaters are unpublished data from Cambridge and results of D. Hilton (personal communication). The south-west England granite data were obtained from a Hot Dry Rock experiment⁶.

The observed 50-fold variation in the R/R_a values of helium sampled from the UK and southern Rhine Graben raises the question of what factors control the transport of mantle-derived helium through the continental crust. The most obvious variables in the regional geology of the areas investigated are the heat flow, the presence of active faults associated with major rift structures, and the timing of the last volcanic episode within the area.

All helium results reported here from the mainland UK and others from south-west England⁶ and Lincolnshire (D. Hilton, personal communication) are from sites where the heat flow has been measured. Heat flow varies from $\sim 125 \text{ mW m}^{-2}$ in south-west England to $\sim 65 \text{ mW m}^{-2}$ in Lincolnshire⁷. R/R_a , however, remains close to 0.03 and shows no dependence on heat flow. This is the relationship to be expected in an old stable region where variation in heat flow depends largely on local variation in heat production in the crust. Although such variations in the abundance of U and Th do affect the heat flow they do not affect R/R_a , but only the total abundance of radiogenic helium. The heat flow in both the North Sea and the Rhine Graben is believed to be affected by deep fluid circulation⁸ and in the North Sea seems to vary with depth but in both areas may locally exceed 100 mW m^{-2} .

The igneous history of the three areas under discussion shows marked differences. Apart from Lundy in the English Channel, the southern part of the British Isles has not been affected by post-Mesozoic igneous activity. The same is probably true of the North Sea. The Rhine Graben, however, has a long history of activity⁹ and late Tertiary eruptions have occurred in the northern part of the Graben near the Eifel (11 Myr) and about 100 km south of Strasbourg at Kaiserstuhl (13 Myr). The highest R/R_a values came from localities close to the Kaiserstuhl. The whole of the Rhine Graben is seismically active today although the boundary faults close to which our samples were collected are relatively quiet; the greater part of the activity occurs away

from the margin fault, either just outside the Graben or clearly within it¹⁰. This difference has been tentatively attributed to the presence of fluids on the main boundary faults¹⁰. The Rhine Graben seismicity appears to weaken in intensity northwards and the Central Graben of the North Sea is nearly aseismic. The most intense seismic activity occurs in the Kaiserstuhl area where the highest R/R_a values are measured. All those samples that contain a component of mantle helium were collected near major faults. There is some suggestion that the highest mantle contributions occur where there is strongest activity. The very highest values come from an area of young igneous activity and high seismicity.

The importance of deep faults is that they are likely to provide transport paths for He through the brittle upper part of the crust which is too cool for diffusion to be effective. Elsewhere³ it has been suggested that the mantle-derived helium component associated with the high-temperature geothermal reservoirs at Larderello in northern Italy, has been advected to shallow ($< 6 \text{ km}$) levels in the crust by magma migration. In the Larderello region particularly, there is no recent volcanic activity at the surface, but a variety of lines of evidence indicate that a shallow ($\sim 7 \text{ km}$)-depth magma body is present and this is consistent with the large component of mantle helium observed near the surface. Similarly, in the southern Rhine Graben, it is possible that mantle helium is brought to comparatively shallow levels in association with magmas, and the faults act as channels for its escape to the surface. If it is real, the much smaller mantle helium component recognized in the North Sea may suggest either incompletely sealed deep fault or even possibly a signal of the onset of renewed activity.

The degradation of organic matter in sediments will be accompanied in space and time by the generation of radiogenic helium, which for typical sediments should have $R/R_a \approx 0.02$. Natural gases that have been sampled from the UK mainland and shelf have associated helium which is close to this value, consistent with a conventional source for the bulk of the hydrocarbons. Several of the North Sea gas samples have R/R_a values that are substantially higher than the expected radiogenic production ratio, which, if interpreted as an addition of a mantle-derived component, places an upper limit of 1% on the mantle helium component. In the case of the CH_4 -rich natural gases that border the Sea of Japan¹¹, the mantle-derived helium component may exceed 50%. Whether or not this might imply a volumetrically significant contribution of mantle CH_4 depends critically on the mantle $\text{CH}_4/^3\text{He}$ ratio and its modification during transport through the crust. On the basis of available observations on the volatile phase associated with oceanic basalts and mantle xenoliths^{12,13}, CH_4 must be considered as a minor species, far subordinate to CO_2 . Only if the dominant mantle-derived C species is CH_4 in continental regions, and $\text{CH}_4/^3\text{He} > 10^{10}$ is this source likely to be significant. Whereas there is the possibility of a trace of mantle-derived component in UK hydrocarbons, particularly close to the axial graben of the North Sea, there is no reason at present to conclude that the major gas CH_4 has anything but a conventional biogenic origin.

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Relics of H₂O fluid inclusions in mantle-derived olivine

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In olivine of upper mantle derivation, CO₂ is the most commonly reported species of fluid¹. Despite its great importance in the upper mantle², H₂O has rarely been reported (refs 3, 4 and E. Roedder, personal communication). Here we report that possible relics of H₂O-rich fluid inclusions have been found in olivine of dunite from the alpine-type Iwanai-dake complex, northern Japan. These inclusions now consist mainly of serpentine and brucite, which may be the products of the reaction, olivine + H₂O = serpentine + brucite, in a closed system. The abundance of such inclusions suggests that the Iwanai-dake complex may have been derived from the upper mantle in conditions in which some free H₂O was available, such as the mantle wedge above a subduction zone.

The Iwanai-dake peridotite complex was emplaced in the southern part of Kamuikotan zone, located at the central zone of Hokkaido. The Kamuikotan zone is characterized by the tectonic mixture of high-pressure (jadeite-glaucophane type) and ocean-floor type low-pressure metamorphic rocks⁵. An ophiolite complex with the basal harzburgite was found at Horokanai⁶, the northern part of the Kamuikotan zone, and was interpreted to be a slice of the Mesozoic oceanic lithosphere which had been consumed to give rise to the collision between the Eurasian plate and the North American plate (the Kurile arc) in the Miocene⁷. However, large quantities of ultramafic rocks, especially those at the southern part of the Kamuikotan zone including the Iwanai-dake complex, constitute, not ophiolitic sequences, but isolated peridotite complexes. The Iwanai-dake complex^{8,9} is composed mainly of roughly layered dunite and harzburgite of upper mantle origin, with very small amounts of chromitite and pyroxenites. Application of the olivine-spinel geothermometry and geospeedometry¹⁰ gives an initial equilibrium temperature of 810 °C (or a little higher) from which the complex had cooled by 200 °C at the rate of 10⁻²–10⁻⁴ °C yr⁻¹. The peridotites around Iwanai-dake are almost wholly devoid of serpentinization and the degree of serpentinization increases abruptly towards the margin of the complex, indicating the post-emplacment nature of the serpentinization. Dunite and harzburgite are strongly magnesian and contain olivines of Fo₉₅₋₉₁ and Fo₉₃₋₉₁, respectively, characterized by exsolution lamellae of chromian spinel⁸.

Olivine is also characterized by many tiny inclusions, which form arrays in thin section (Fig. 1). These inclusions were examined in a particular sample of dunite (IW-8407) composed of equant coarse (up to 1 cm diameter) olivine (Fo₉₄₋₉₃, Fo₉₃ on average) and euhedral chromian spinel (Cr/Cr + Al atomic ratio, 0.75).



Fig. 1 Photomicrograph of inclusions in olivine, taken in plane-polarized light. Inclusion arrays are sharply cut by the post-emplacment serpentine veins. Scale bar, 50 µm.

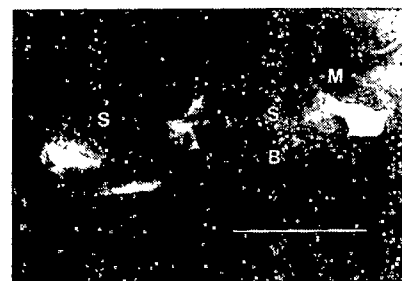


Fig. 2 Transmission electron micrograph of an inclusion containing serpentine, brucite and magnetite (indicated by S, B and M, respectively). Note that the inclusion is completely surrounded by host olivine. Scale bar, 1 µm.

Some inclusions crosscut the kink band, suggesting that they formed after recrystallization of the host olivine. The shape of an individual inclusion is typically columnar. The inclusion arrays are clearly cut by post-emplacment serpentine (PES, chrysotile-lizardite), but do not cut or distort the fine texture of the serpentine vein. An individual inclusion can be shown to be isolated in the host olivine (Fig. 1).

The ion-thinned foils were observed and analysed in JEOL 200-CX electron microscope¹¹. The inclusion (Fig. 2) is completely surrounded by the host olivine and has a clear-cut outline of hexagonal shape, forming a sharply faceted negative crystal (Fig. 2) elongated parallel to [100] of the host olivine. Electron diffraction (Fig. 3a, b) and semi-quantitative analysis revealed that serpentine (S in Fig. 2), which forms bundle and radial fibrous aggregates, and brucite (B in Fig. 2), which is a rhomboid extended along the inclusion wall, are the main constituents of the inclusion. Notably, the volume ratio of serpentine to brucite is approximately 4 (Fig. 2). Species of the serpentine mineral could not be distinguished. A minute grain of Fe-Ni alloy or magnetite was always found (Fig. 2).

Because the present inclusion is completely surrounded by the host olivine, it is unlikely to have formed as a result of an external source of water at temperatures low enough for serpentine to remain stable. It is also improbable that the inclusion of hydrous minerals represents an early stage of the PES of the rock, because the inclusion arrays do not affect the fine texture of the serpentine vein (Fig. 1). We know of no plausible mechanism for the formation of such inclusions in olivine at the even lower temperature that would prevail after the PES. Nor is the array of inclusions a partially healed veinlet of serpentine and brucite, as no traces suggestive of the hydration-dehydration stage before the PES (for example, the paragenesis of olivine + magnetite or ferritchromit) could be found. The most appropriate interpretation is that, when the temperature (and possibly pressure) decreased, the water vapour trapped before the PES reacted with the surrounding olivine to form hydrous minerals via reactions such as $2\text{Mg}_2\text{SiO}_2$ (olivine) + $3\text{H}_2\text{O} =$

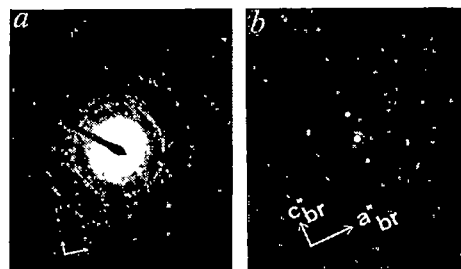


Fig. 3 Electron diffraction patterns of serpentine (a) and brucite (b). Debye rings and streaks observed in a can be indexed as reflections of serpentine. A net-like pattern in the lower left-hand of a (indicated by arrows) is that of *hk0* of host olivine (shorter and longer arrows, *b** and *a**, respectively).

$\text{Mg}_3\text{Si}_2\text{O}_5(\text{OH})_2$ (serpentine) + $\text{Mg}(\text{OH})_2$ (brucite)¹². This reaction gives a volume ratio of serpentine to brucite of ~ 4.4 (ref. 13), which is consistent with that observed (~ 4). Since magnesite was not found in the inclusions examined, the $\text{CO}_2/\text{H}_2\text{O}$ ratio of the fluid must have been very low.

The present relic H_2O -rich fluid inclusions show the characteristics of so-called secondary inclusions¹, which implies that they were formed by healing of the fracture in olivine which had been filled by water vapour. The inclusion was formed at temperatures $>400^\circ\text{C}$, below that at which the ordinary serpentinization had occurred¹², and after the main deformation stage, which occurred at high temperatures. It is probable that the Iwanai-dake complex was derived from the relatively low-temperature upper mantle where free H_2O was available. This situation could be most easily achieved at the mantle wedge^{14,15}, especially near the leading edge, above a downgoing slab, where free H_2O in addition to CO_2 may be generally available. For example, peridotite xenoliths from the Ichinomegata crater (400 km on the continent side of the Japan Trench)^{16,17}, northern Japan, derived from the hydrated mantle wedge beneath the North-East Honshu Arc¹⁸, are the only known mantle materials containing H_2O -bearing fluid inclusions^{3,4}. In contrast, other peridotite xenoliths containing almost pure CO_2 fluid inclusions were derived from the upper mantle free of underlying subduction zones⁴. Olivine in peridotites of the mantle wedge, if slowly cooled to low temperatures, would have inclusions of serpentine-brucite, serpentine-brucite-magnesite, serpentine-talc-magnesite and talc-magnesite-enstatite, depending on the $\text{H}_2\text{O}/\text{CO}_2$ ratio of the fluid¹⁹. Peridotites in which H_2O -rich fluid inclusions constitute the leading edge of the mantle wedge

(or the fore-arc mantle) may be emplaced as ophiolites or alpine-type masses²⁰ like the Iwanai-dake complex, during the arc-arc collision in Hokkaido in the Miocene⁷. Peridotites of the typical fore-arc ophiolite, such as the Papuan Ultramafic Belt²¹, would be expected to contain olivine with serpentine-brucite inclusions.

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Improved kerogen typing for petroleum source rock analysis

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The chemical characterization of kerogen (typing) is a routine part of petroleum exploration programmes¹. Traditionally, kerogen has been characterized on the basis of microscopy² and bulk chemical methods³, procedures derived from the coal characterization literature^{1,4}. These methods do not today, however, provide adequate resolution of the complex mixture of kerogen components encountered in source rocks from petroleum-bearing sedimentary basins⁵. Here we report an improved classification approach for kerogens at all maturity levels. The method is based on a quantitative pyrolysis-gas chromatography approach using a polymeric internal standard, polymethylstyrene, to directly determine the absolute concentrations of specific kerogen pyrolysis products. In particular, cross-plots of normal hydrocarbon, aromatic hydrocarbon and total hydrocarbon yields of kerogen pyrolysis are used to recognize eight separate nodes of kerogen composition.

Two major factors control the petroleum potential of any sediment at any maturity level: (1) the quantitative fraction of sediment that contains potential petroleum-related chemical structures, and (2) the efficiency of expulsion of these petroleum products from the source bed^{1,5}. The maturity level of organic matter in a sediment simplistically reflects its extent of reaction along the reaction coordinate system representing reaction converting sedimentary organic matter (immature) to oil (mature) and ultimately methane and graphite (post-mature).

The fraction of the sediment that contains catagenetically stable petroleum-related structures is correlated closely with the position of the sediment on the reaction coordinate system. Pyrolysis-gas

chromatography (pyrolysis-GC) is widely used both to elucidate the structure of kerogens^{5–9} and to type them according to chemical properties and physical characteristics^{5,6–14}, with a view, among other applications, to determining catagenic petroleum yield and composition¹. However, conventional pyrolysis-GC fingerprinting of kerogens is a rather subjective process and cannot easily resolve differences between the continuous series of mixed type II and type III kerogens often found in near-shore marine depositional settings. Ideally, kerogens should be characterized at the same molecular level, as is possible with rock extracts and crude oils; thus, full quantitation of all pyrolysis products (as may be possible with calibrated pyrolysis-mass spectrometric methods) is desirable. Here, we have used pyrolysis-GC to quantitate normal hydrocarbon yields from kerogens on pyrolysis, and these data are used to better define kerogen type and, hence, petroleum yield and type.

Normalized abundances of pyrogram (pyrolysis-gas chromatogram) components were obtained on a per-kerogen or per-carbon basis, using a polymethylstyrene internal standard that was added and mixed in dichloromethane solution with the extracted kerogen. Pyrolysis-mass spectrometry and thermogravimetric analysis showed that the standard polymer decomposed during pyrolysis, at temperatures well below the maximum temperature (800°C), to yield a monomer (methylstyrene) as predominant product. The use of a polymeric internal standard is necessary due to the nature of the pyrolysis system used, in which sample loss during instrument set-up sometimes occurs and where a volatile standard would be lost non-quantitatively from the sample before pyrolysis. The device is described in Fig. 1 legend and is documented elsewhere¹². Polystyrene has been used in quantitative pyrolysis-GC studies of soils¹⁵ but the common presence of styrene (the pyrolysis product of polystyrene) as a kerogen pyrolysis product would interfere with the use of polystyrene here. Methylstyrene, while a minor component of some coal maceral kerogen pyrolysates^{12,13}, provides a component that is generally at a low concentration in most pyrolysates, and which elutes chromatographically away from other peaks of interest in pyrograms of most source rock kerogens.

Fig. 1 Normalized abundance (by weight of kerogen) of aliphatic and aromatic hydrocarbons in low-maturity kerogen pyrolysates. The diagram represents a cross-plot of the abundance of C_7 - C_{25} normal hydrocarbons (alkenes plus alkanes) and of C_6 - C_8 alkylaromatic hydrocarbons (benzene, toluene, ethylbenzene, xylenes, styrene) obtained by pyrolysis at 800 °C for 20 s of up to 500 μ g of solvent-extracted kerogen. \square , Type I: alginites from Australia. Δ , Type II: Miocene, California; Triassic, North Sea; Jurassic, Paris Basin; Jurassic, North Sea; Tertiary, Alaska; Carboniferous, UK. \circ , Type III: Jurassic, North Sea; Miocene, Indonesia; Cretaceous, Canada. \times , Type IV: Carboniferous, UK.

Methods. The kerogens were pyrolysed on a platinum filament in flowing helium on a Chemical Data Systems 120 Pyroprobe, modified for use with capillary GC columns¹². Kerogen/poly-methylstyrene mixtures were placed on the cleaned pyrolysis filament in methanol suspension via a glass capillary tube; the methanol was evaporated before pyrolysis. The procedure used is described in ref. 12. GC analysis was performed via a splitter system, the pyrolysate being separated on a 50-m SP2100 capillary column programmed from 40 to 300 °C at 4 °C min⁻¹. Hydrocarbons were quantitated using a methylstyrene internal standard generated during the pyrolysis process from polymethylstyrene admixed with the kerogen. Automated peak area integration of the pyrograms was done on a microcomputer. Replicate analyses of aliquots of the samples allowed determination of the reproducibility of the method (error bars represent 2 σ variation about the mean). The kerogens used were all of maturity less than that equivalent to a vitrinite reflectance level of 0.8%. One standard unit approximately equals 0.25%, by weight, of kerogen.

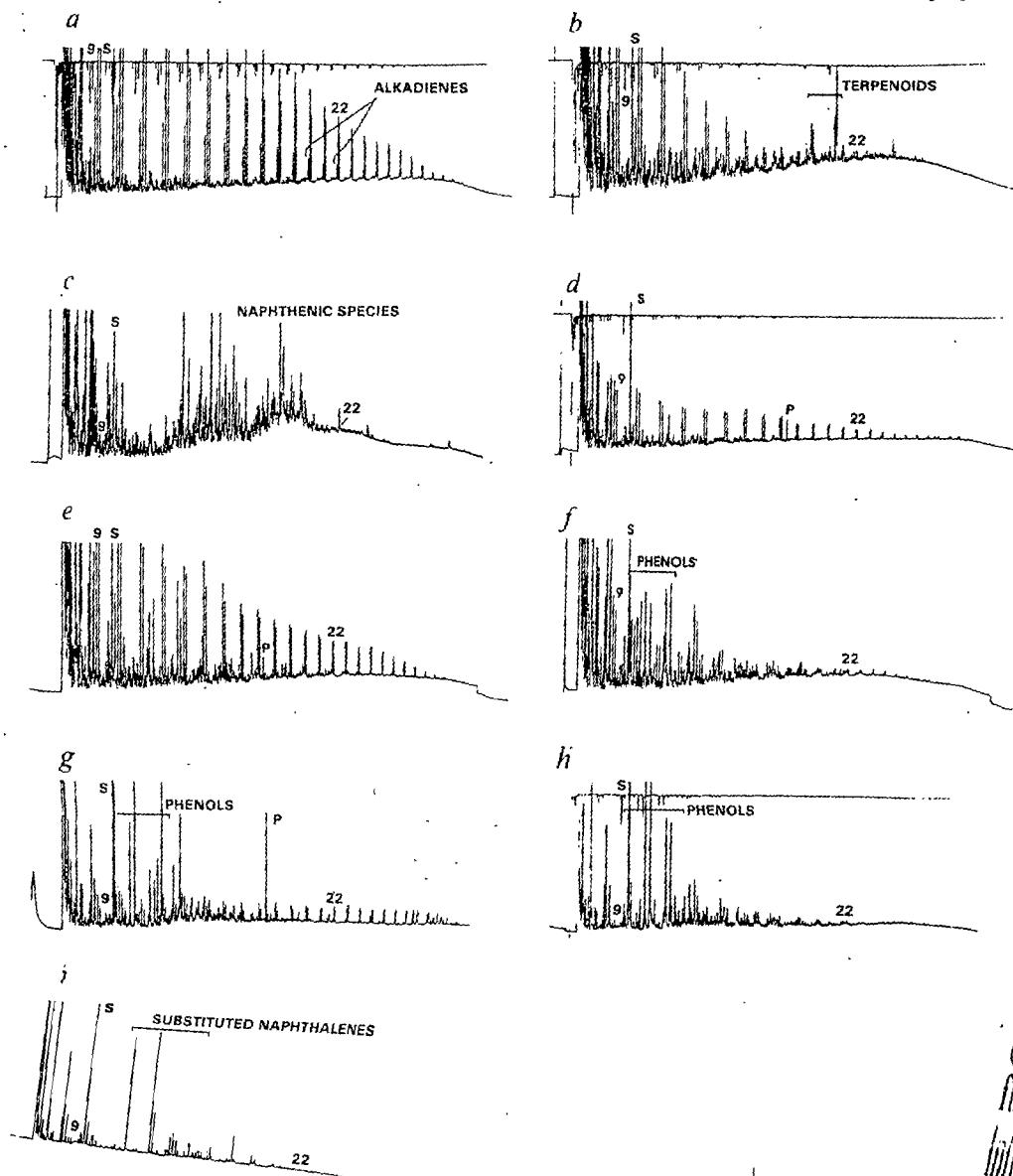
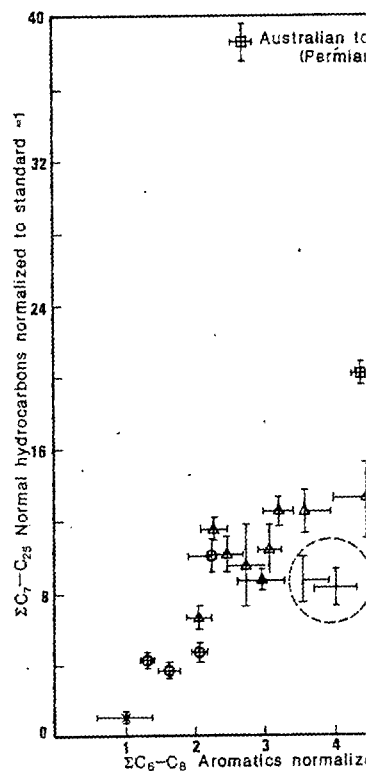


Fig. 2 Prit defined by shows cap kerogens, <0.8%, equivalent.

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Internal standard and the C₉ (9) and C₂₂ and alkanes and P, pre-i-e indicated. Column condition Fig. 1.

Figure 1 shows the absolute yield of aromatic (expressed as total benzene, toluene and C_8 aromatics) and normal hydrocarbons (C_7 - C_{25} n -alkenes plus n -alkanes) for a variety of low-maturity kerogens. Component abundances were determined by automated peak recognition and integration on a laboratory microcomputer system. Interestingly, the absolute normal hydrocarbon content of several of the type II and III kerogens is similar. In general, it is the higher naphthenic contribution from marine organic matter that provides the increased hydrogen indices of marine kerogen systems. Replicate analysis reveals that some approach to accurate quantitation of kerogen pyrolysis products can be made rapidly in this manner and that the method may prove applicable to more general quantitative characterization of complex geopolymers.

While fingerprint approaches have been successfully applied to kerogen typing using pyrograms^{6,8-10,12,13}, it is the lack of high-resolution quantitative data that has precluded typing kerogens to the same level of resolution with which source rock bitumens or oils are characterized¹. Figure 2 shows pyrograms of several kerogens which we believe represent distinct molecular assemblages at low maturity levels. Figure 3 shows a cross-plot of a hydrogen index parameter (determined from elemental analysis data; compare with ref. 16) and the total organic carbon (TOC)-normalized abundance of C_9 - C_{30} normal hydrocarbons estimated from peak height data. It is the relative positions of the kerogens on this diagram, the pyrogram fingerprint character, and a knowledge of the petrographic properties of the kerogens that allow this more refined subdivision of the classical Tissot typing scheme³. The inclusion of petrographic and chemical data in the characterization of source rock kerogens is widely practised within petroleum geochemical circles¹ and is a critical requirement when quantitative assessments of source rocks are required in terms of volumes of oil sourced from a source bed⁵.

By directly determining the total reservoir of specific pyrolysis elements present in the kerogen at any maturity level in the manner described above, direct assessment of actual petroleum potential for any kerogen system becomes possible on a molecular level. Further, this method allows the discrimination of kerogens hitherto grouped together on the basis of elemental analysis or hydrogen/oxygen indices. Thus, in Fig. 2g, h, the two type III kerogens analysed, while petrographically vitrinite-dominated, are clearly different in detailed composition. Vitrinites from the deltaic system (Fig. 2g) have appreciable quantities of normal hydrocarbon precursors in the system while the vitrinite from a coal swamp environment (Fig. 2h) is paraffin precursor-depleted. In assessing hydrocarbon-generating potential for a kerogen assemblage, it is frequently difficult to deduce actual potential from bulk chemical analyses as many detailed compositions, some capable of generating components of high molecular weight, are possible for any given bulk chemical analysis.

The detailed composition of kerogen pyrolysates as a function of type has been extensively reviewed before⁸⁻¹³ with regard to normal hydrocarbon and hydrogen index variation, as summarized below and in Fig. 3. Type I kerogens (Figs 2, 3) are generally characterized at immaturity with high hydrogen indices but the thermal degradation products may be highly paraffinic (Ip), mixed paraffinic/naphthenic (Ipn), or naphthenic (In), depending on source facies^{6,12} (examples are shown in Fig. 2). Type II kerogens with intermediate hydrogen indices and relatively low paraffin contents (IIpn) are characteristic of kerogens from marine source beds containing dominantly amorphous organic matter derived from algal and bacterial sources². Kerogens from many clastic 'super-source rocks' fall into this category, examples being kerogens from the Kimmeridge Clay Formation (North Sea basins), Lower Cretaceous sediments from the North Slope basins of Alaska, Miocene sediments from the Tertiary basins of southern California, and Lower Jurassic sediments from the Paris Basin. Type II kerogens with intermediate hydrogen indices but high paraffin precursor contents (IIp) are generally associated with land plant-derived exinite-rich systems.

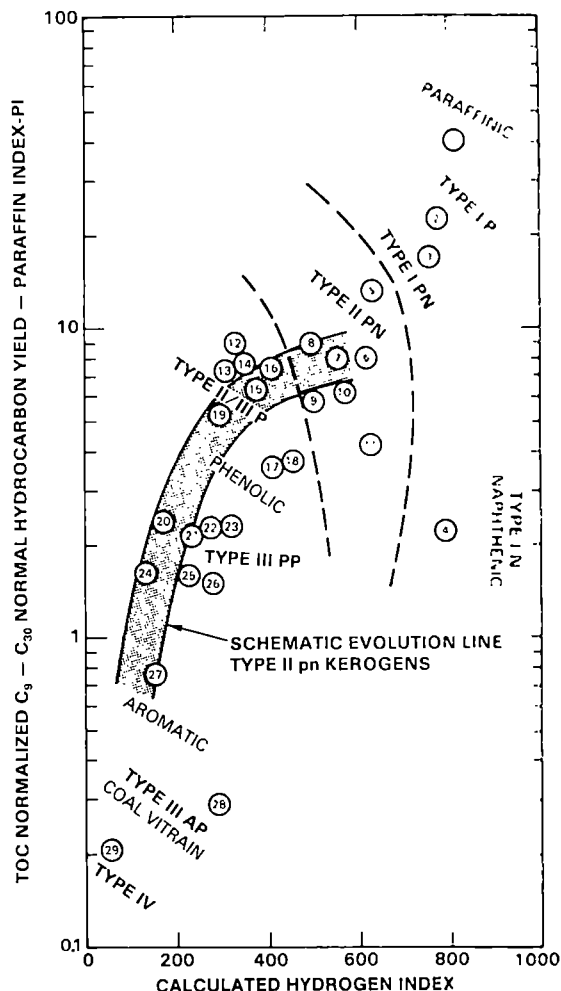


Fig. 3 Paraffin index/hydrogen index cross-plot. The figure shows a cross-plot of the summed abundance of the C_9 - C_{30} n -alkenes plus n -alkanes determined from pyrogram peak height data plotted against a computed hydrogen index derived from elemental analysis data in the manner of Orr¹⁶ (Rockeval units = mg hydrocarbons per g TOC). Although not a true hydrogen index determined analytically, this approach does allow sample comparison on a common matrix effect-free basis. Paraffin index is based on the pyrolysis-GC unit = 0.25% by weight. Representative kerogen types: Ip, torbanite (*Botryococcus*) kerogens, rich in alginite (samples 1, 2); Ipn, tasmanite (alginite)-rich kerogens (sample 3); In, resinite-derived materials (sample 4); IIpn, amorphinite-rich kerogens from source rocks containing marine organic matter (samples 5-11); II/IIIp, exinite-rich kerogens (samples 12-16); II/IIIpp, Carboniferous sporinites (samples 17, 18); IIIpp, vitrinite-rich kerogens from marine/deltaic shales (samples 19-26); IIIap, vitrinite-rich kerogens from coal swamps (samples 27, 28); IV, inertinite-rich kerogens (sample 29). p, Paraffin-dominated pyrogram; pn, paraffin/naphthene-dominated; n, naphthene-dominated; pp, phenol/paraffin-dominated; ap, aromatic hydrocarbon/phenol-dominated.

As described above, immature type III kerogens may have paraffin contents ranging from high (IIIp) to almost zero (IIIap); type IV sediments are characterized solely by lean aromatic hydrocarbon-dominated pyrolysates¹².

Molecular approaches to kerogen typing are still in their infancy, but together with bulk chemical and petrographic characterization methods, they provide an improved method for examining the chemically and physically complex mixtures that represent the mixed type II/III kerogens of many near-shore marine settings. It is these mixed assemblages that are the least well-defined using established approaches, yet are the most common organic facies found in many of the world's basins currently being explored. The general approach outlined here,

while far from resolving this critical problem, is believed to be a necessary step in the development of new high-resolution quantitative methods for source rock kerogen characterization.

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Magnetostratigraphy and palynology demonstrate at least 4 million years of Arctic Ocean sedimentation

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It has long been recognized^{1,2} that the Arctic Ocean has a major role in regulating the timing of Northern Hemisphere glaciations, global heat exchange and ocean circulation. Several recent studies³⁻⁷ have attempted to interpret the palaeoclimatic record in sediment cores from the Arctic Ocean, but conflicting ages have been reported for palaeoclimatic events dated by different methods³⁻⁶. To establish a consistent chronology for cores from the central Arctic Ocean, we performed detailed palaeomagnetic and palynological studies of three cores collected⁷ near the crest of the Alpha Ridge, and obtained radiocarbon dates from planktonic foraminifers isolated from 1-cm-thick intervals. Magnetic inclination values for these cores clearly record normal and reversed-polarity intervals that apparently correspond to the Brunhes and Matuyama magnetochrons; the longest core also includes polarity intervals typical of the Gauss and Gilbert magnetochrons. This chronology is confirmed by the first and last occurrences of pollen and dinoflagellate species that can be correlated with European^{8,9} and DSDP¹⁰⁻¹² chronostratigraphies dated by standard biochronological methods. Micromass radiocarbon dates for foraminifers indicate a sedimentation rate of ~1 mm per 1,000 yr. We believe that these data confirm a long (>4 Myr) chronology for the Alpha Ridge cores, the lithostratigraphy of which can be correlated over extensive areas of the western Arctic Ocean²⁻⁷, and more important, provide dinoflagellate and palynomorph data that indicate the validity of early chronology.

Previous chronostratigraphic studies of Arctic Ocean sediments have been hampered by (1) problems of core handling and storage prior to sampling⁶; (2) incomplete reporting of magnetic data^{3,5,6}; (3) lack of dateable calcareous and siliceous microfossils in the tertiary sediments⁵; and (4) uncertain validity of U/Th dates from hemipelagic muds³.

Our cores were obtained in conjunction with high-resolution seismic profiles from the 1983 CESAR ice station⁷ and they

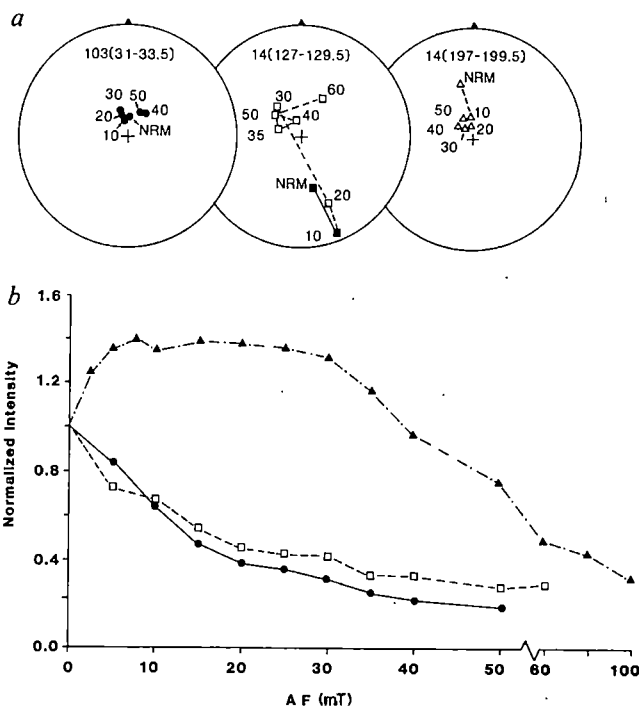


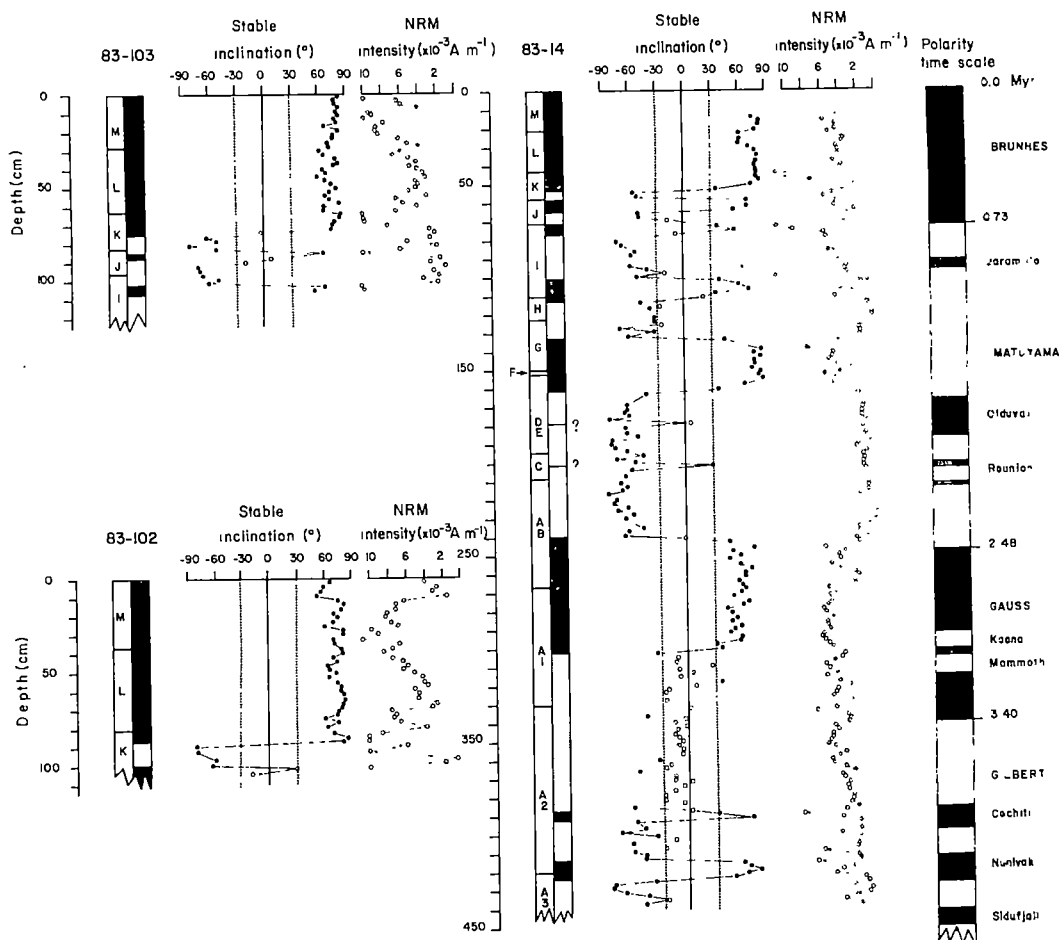
Fig. 1 Examples of directional response to AF demagnetization; open symbols indicate negative inclination, closed symbols indicate positive inclinations (a) and the normalized intensity response to AF demagnetization of these samples (b) where ● = 103 (31-31.5 cm), □ = 14 (127-129.5 cm), △ = 14 (197-199.5 cm).

were stored upright and prevented from freezing before laboratory studies. Two gravity cores, CESAR 102 (85°38.1' N, 111°07.1' W) and CESAR 103 (85°38.1' N, 111°07.0' W), and one long piston core, CESAR 14 (85°50.8' N, 108°21.6' W) were selected for study to ensure that a complete sequence of the surface sediments would be sampled (piston cores rarely recover the surface sediment layer). Cores were split 2-6 months after their recovery, and the sediments are free of post-coring disturbances due to desiccation, oxidation and previous sampling. Palaeomagnetic samples were taken using a plastic cylinder of 2.5 cm diameter¹³. After palaeomagnetic measurement, each sample was split at right angles to the vertical core axis, and the sediments were processed for palynological studies using HCl, HF and sieving methods¹⁴.

Palaeomagnetic measurements of the samples were made¹³⁻¹⁵ using a Schonstedt DSM-1 Digital Spinner Magnetometer. The intensity of the natural remanent magnetization (NRM) ranged from $0.1 \times 10^{-2} \text{ A m}^{-1}$ in normally magnetized samples to $0.5 \times 10^{-3} \text{ A m}^{-1}$ in reversely magnetized samples. Further magnetization measurements were made after alternating field (AF) demagnetization steps until the intensity values were reduced to <15% of the NRM, which usually occurred between 30 and 40 mT. As a precaution against excess demagnetization, however, all samples were stepwise demagnetized at 2.5, 5, 7.5, 10, 15, 20, 25, 30, 35 and 40 mT, with a few samples further demagnetized to 100 mT. Most normal and reverse stable remanence directions had associated precision parameters, k , with corresponding semi-cones of confidence, α_{95} , being less than 5°.

Figure 1 shows examples of the normalized intensity and directional response to AF demagnetization. Some of the remanent magnetization directional scatter may be caused by using the 'push-in' sampling method¹⁶. Normally magnetized core sections typically showed a steady decrease in intensity with demagnetization, accompanied by little directional response. Reversely magnetized sections typically showed an initial positive NRM that gradually changed to negative values with removal of the overprinted viscous remanent magnetization (VRM) after treatment at 10-20 mT. The steady decrease in normalized intensity suggested that the reverse stable remanence

Fig. 2 Downcore plots of stable inclinations (●) and NRM intensities (○). ○, Transition zones; □, samples in which a stable phase of magnetization was not achieved. A3–M, lithostratigraphic units⁷. Summary polarity logs for each core are based on all polarity changes that exceed +30 or -30° on their stable inclination; values of <30° are indicated as transitional. Polarity time-scale is from Harland *et al.*¹⁸.



intensity was weaker than the VRM. Some normally magnetized samples and about 20% of the reversely magnetized samples showed a progressive increase of intensity on treatment at 10 or 15 mT, followed by a steady decrease on demagnetization at 50 or 100 mT. Removal of the VRM caused little directional response in these samples.

Plots of stable inclination versus depth (Fig. 2) clearly reveal normal and reversed-polarity chrons like those observed in global magnetostratigraphies^{17,18}. The Brunhes-Matuyama boundary occurs around 71–77 cm depth in CESAR 103, at 85–90 cm in CESAR 102 and at 50–55 cm in CESAR 14. In all cores, this transition is sharp and is associated with a decrease in intensity of $0.8 \times 10^{-3} \text{ A m}^{-1}$ in the middle of lithofacies K. The Matuyama-Gauss transition is identified from the 239–244 cm depth in CESAR 14. Within the Matuyama reverse chron, four major positive subchrons occur in CESAR 14 and two are found in the gravity cores. All polarity changes are associated with large changes in intensity. The most prominent subchron (135–161 cm in CESAR 14) is tentatively correlated with the Olduvai normal event. The two short-lived positive events just below the Brunhes-Matuyama boundary are tentatively correlated with the Jaramillo event. The event at 102–109 cm depth in CESAR 14 may be either a split Olduvai subchron, with the lower part occurring at 135–161 cm, or the Gilsa event. The transition from Gauss normal to Gilbert reverse-polarity chrons is inconspicuous in CESAR 14. The stable inclination oscillated between 60° and 75°, then a 70° shift occurred, below which few samples showed clear stable remanence directions. The Gauss-Gilbert transition is therefore tentatively placed at ~300 cm depth.

Inclination values for the Brunhes and Gauss positive polarity chrons in CESAR 14 (Fig. 3) were strongly skewed towards high positive NRM values and showed little displacement with stepwise demagnetization. NRM inclinations in the reversed polarity Matuyama chron showed a slight skewness towards positive

NRM values, gradually displacing towards the negative values to form a well-defined peak at -45° to -75° by 40 mT. This behaviour demonstrated the successful removal of low-coercivity downward normal components, and the isolation of high-coercivity reversed-polarity components. It is clear that the viscous component is not totally removed before 30–40 mT demagnetization steps, and that samples show progressive directional response with each demagnetization step (Figs 1, 3). In most previous palaeomagnetic studies^{19–21} of Arctic Ocean sediments, samples were routinely demagnetized at 5–15 mT. The CESAR cores show that over 80% of the samples required demagnetization to 40 mT. Hence, insufficient demagnetization may account for the considerable scatter observed in inclination values reported previously.

Palynological studies of CESAR 103 and 14 were made on the samples used for palaeomagnetism measurements. Detailed data for CESAR 103 and initial results for CESAR 14 are documented elsewhere²²; systematic descriptions of the dinocysts (dinoflagellate cysts) are given by Mudie¹¹. The lithostratigraphy (facies A3 to M) and regional core correlations are documented elsewhere⁷. Here (Fig. 4) we show only the ranges of selected dinocyst, pollen and spore taxa in CESAR 14. Some notable similarities between the ranges of the marine dinocysts and those of terrestrial pollen and spores are evident: (1) The base of facies A2 is marked by the last appearance datum (LAD) or disappearance of two dinocyst taxa and five pollen/spore taxa, and one dinocyst taxon and one pollen taxon have their first appearance datum (FAD) in this interval. (2) Between the top of facies AB and C, three dinocysts and one pollen taxa have their LAD. (3) Between the top of facies G and the base of I, one dinocyst taxon disappears, and six dinocyst species and three pollen/spore taxa appear or reappear; the base of the Gramineae/Cyperaceae acme zone is found near the top of facies I. (4) In facies J to K, the calcareous dinoflagellate¹¹, *Thoracosphaera arctica* Gilbert and Clark, and several

in the early Pleistocene. These occurrences are in accord with our magnetochronology (Fig. 2). No FAD have been reported previously for *Picea mariana*, *Polytrichum* or *Peridinium faeroense* Dale, but at Site 611, these boreal taxa first become common at the onset of cooling at the Plio-Pleistocene boundary. The range of *T. arctica* has been dated as early Pleistocene in a number of other Arctic Ocean cores^{9,24} using palaeomagnetism and U/Th dating methods, and *O. centrocarpum* has an early Pleistocene acme zone in the Bering Sea.

(4) **Facies J to K.** At Site 611 and in the Bering Sea, *Spiniferites frigidus* has its FAD at or near the early/late Pleistocene boundary, which agrees well with our data for CESAR 14. In contrast, the FAD of *Votadinium calvum* and *Impagidinium patulum* and the base of the Gramineae/Cyperaceae acme zone occurs in the late Pliocene or earlier in the North Atlantic, *Nyssa* has its LAD in the late Pliocene of Europe and eastern Canada, and *Pseudotsuga* disappears at the top of the Cromerian Stage (~1 Myr) in Europe. Overall, correlation is not very precise for this interval in CESAR 14, but the palynomorphs definitely indicate a minimum middle Pleistocene age rather than the shorter (0.2 Myr) chronology proposed by Sejrup *et al.*³.

(5) **Facies L.** In Europe, *Juglans* has its LAD at the Holstein/Saalian boundary (~0.5 Myr), and Site 611, the tops of the *Quercus* and *Acer* acmes, and the LAD of *Tsuga* occur in the middle Pleistocene, indicating an age of 0.5 to at least 0.2 Myr, which agrees reasonably well with the magnetochronology proposed for CESAR 14.

Palynological correlation thus provides a chronology for the Alpha Ridge cores which generally supports the magnetochronology shown in Fig. 2. Hence, we conclude that the sedimentation rate on the Alpha Ridge has been very slow, about 1–1.5 mm per 1,000 yr, during the past 4.25 Myr. Further evidence for a very slow sedimentation rate is provided by tandem accelerator micromass radiocarbon dates from planktonic foraminifers (*Neoglobobulimina pachyderma* sinistral) that were hand-picked from the >150- μ m sediment fraction of CESAR 102 at 2–3 cm and 5–6 cm depth. Dates of >30,000 yr BP were obtained for both these samples (laboratory numbers Beta-12230 and Beta-12231, respectively). This slow deposition rate is in accord with previous magnetostratigraphic studies^{2,5,9,19,21}. These data conflict strongly with the short chronology and higher sedimentation rates (1–2 cm per 1,000 yr) obtained from dating by amino-acid epimerization³. In view of the potential importance of understanding the role of the Arctic Ocean in regulating the timing of Northern Hemisphere glaciations^{1,2}, detailed magnetostratigraphic and lithostratigraphic correlations and confirmation of dating using organic walled palynomorphs (which are not susceptible to carbonate or silica dissolution and are thus present in all the lithofacies) are important prerequisites for studying palaeoecological and isotope records from Arctic Ocean cores.

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Fronde-like fossils from the base of the late Precambrian Wilpena Group, South Australia

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Late Precambrian metazoan fossils are at present known from at least 23 different regions in Africa, Asia, Australia, Europe and North America¹. The Ediacara fossil assemblage^{2,3} occurs widely in the Flinders Ranges, South Australia, near the base of the Rawnsley quartzite of the Pound Subgroup. This complex of soft-bodied organisms includes medusoid coelenterates, soft corals related to pennatulids, segmented worms, arthropods and other simple coelenterates^{4,5}. Here I report the discovery of frond-like fossils comparable to those of the Ediacara assemblage more than 3,000 m stratigraphically below the Ediacara Member⁶ of the Pound Subgroup. This discovery supports the placement of the base of the Ediacaran⁵ in South Australia at the boundary of the Wilpena Group and the Umberatana Group, a stratigraphic level which marks the termination of late Proterozoic glaciation in the Adelaide Geosyncline.

The Adelaide Geosyncline (Fig. 1) represents a sedimentary sequence that accumulated in response to a branching network of separate rifts with an overall meridional trend across the middle of the Australian continent^{7,8}. The late Precambrian Wilpena Group, a succession of predominantly terrigenous clastics deposited within the Adelaide geosyncline^{9,10}, rests conformably on glaciogenic sediments of the Umberatana Group¹¹. The Brachina Subgroup is the lower of two upward-coarsening sequences within the Wilpena Group (Fig. 2). A detailed stratigraphic study of this sequence at Hallett Cove, South Australia (Fig. 1), has identified turbidite and storm-dominated shallow marine facies¹². This section correlates very strongly with that of the fossil site.

The fossils reported here are preserved on an upper bedding-plane surface within basal sandstones of the Brachina Subgroup cropping out in Alligator Gorge of the Mount Remarkable National Park in the southern Flinders Ranges (Fig. 1). This sequence passes upward from massive sandstones which intertongue with dolomites of the Nuccaleena Formation, through interbedded shales and fine sandstones, into a thick medium-grained cross-bedded unit known as the ABC Range quartzite (Fig. 2). The upward coarsening and thickening Brachina Subgroup is interpreted to be the result of initial transgression followed by progradation into a sedimentary basin. Sedimentation took place in a storm- and tide-dominated shallow marine environment with an ENE slope into a deeper turbidite basin.

I do not propose to present a detailed taxonomic classification of the metazoan fossil. However, further investigation of the study area will hopefully result in the discovery of more specimens to help with systematics (the specimen has not been removed from the discovery site). The novel fossil may be interpreted as representing organisms which were basically foliate, leaf-shaped (frond) structures, with each preserved specimen lying on one of its two broad sides on a bedding plane within the sandstone. The frond-like structures consist of a

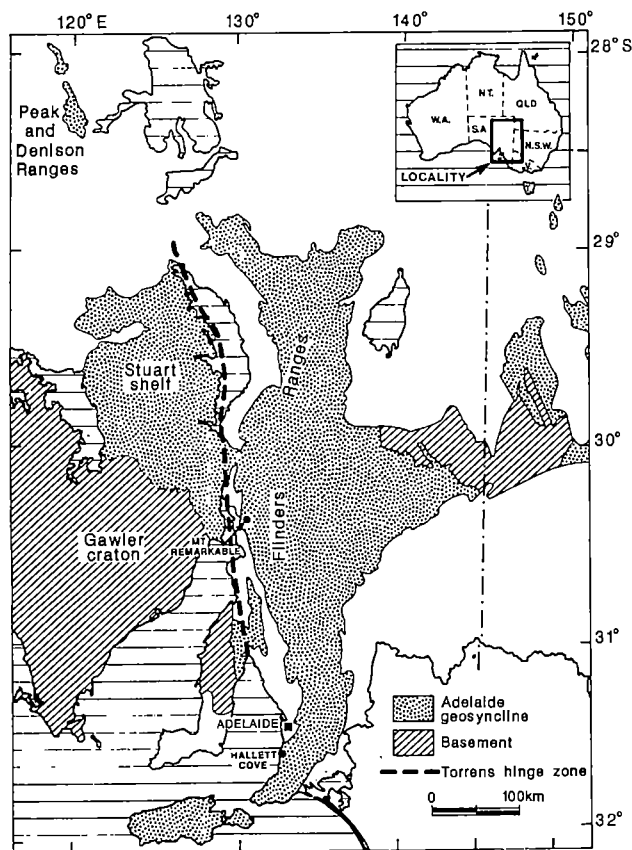


Fig. 1 Outcrop area of the late Proterozoic Adelaide geosyncline. Note the locality of the study area: Mount Remarkable in the southern Flinders Ranges.

relatively narrow median zone from which extend bilaterally opposed leaf-like branches. The specimens show regular spacing of branches, with specimen *b* (Fig. 3) displaying a wider spacing between consecutive branches. A number of incomplete individuals, preserved as impressions on a very thin shale parting in massive sandstone, are thought to have been oriented by rip or turbidity currents flowing in an ENE direction. The specimens overlap and show signs of distortion, making identification of individual outlines difficult. An incomplete frond (a in Fig. 3) is ~30 cm long and 6.5 cm wide, and is composed of about 30 partially or completely preserved branches extending from one side of a broad axial region. The median track is ~20 mm wide and tapers to a point at one end. Large areas of the branches show no systematic curvature, with each branch extending from the median track at an angle of 45°. Each branch is bounded by a well-defined groove. A more complete specimen (b in Fig. 3) clearly exhibits a narrower median track about which numerous lateral branches exhibit bilateral symmetry. The branches appear to extend opposite one another at an angle of 70–85° from the median track and are bounded by evenly spaced repetitive grooves. The specimen is 37 cm long and 9 cm wide.

Comparison of these specimens with those fossils from the Pound Subgroup representing sessile colonial *Cnidaria*¹ indicates that the metazoans of this latest discovery in the Flinders Ranges may belong to one of the two families Pteridiniidae Richter, 1955 and Charniidae Glaessner, 1979, as the fossils consist of a median track from which a leaf-like expansion extends with bilateral symmetry. Members of the former family are rare from the Flinders Ranges. These frond-like fossils of the Ediacara assemblage are considered to be allied to the living members of the Order Pennatulacea^{1,13}. Modern pennatulids (sea pens) are found growing vertically on sandy sea floors¹⁴ with their stalk moored to the substrate by a holdfast. Sea pens are found washed up on beaches, after being torn from their moorings by storm action. Fossils of the genus *Pteridinium*

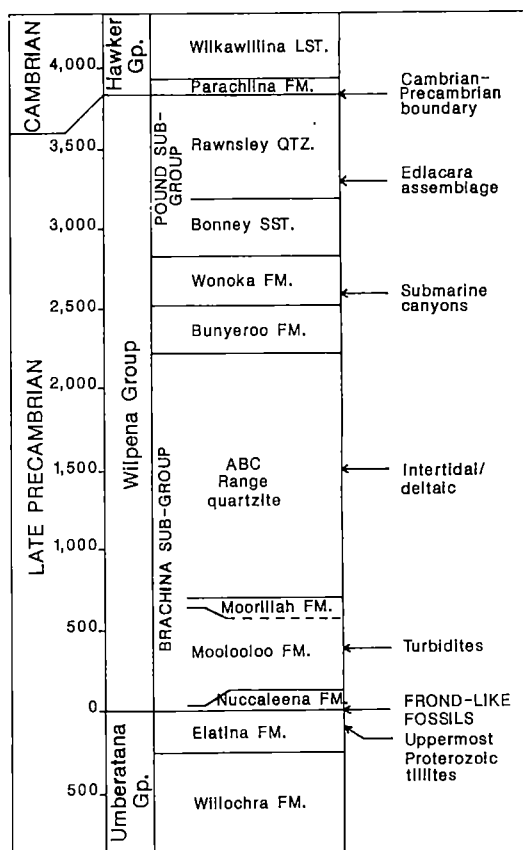


Fig. 2 Stratigraphy of the late Precambrian Wilpena Group in southern Flinders Ranges. LST., limestone; FM., formation; QTZ., quartzite; SST., sandstone.

Gürich are known to occur in storm beds¹⁵. The median track of *Charniodiscus* Ford² extends downwards into a stalk which terminates in a basal attachment disk; lateral branches extend from the median track at an inclination of ~65–85° and bear foliate structures (polyp leaves) divided into ridges (polyp anthostoles). These secondary divisions that occur between the polyp anthostoles of the Charniidae, appear to be rare and minute in the family Pteridiniidae¹. In *Pteridinium*, three ribbed wings may extend from the axis¹⁶. *Pteridinium simplex* is characterized by its incised zig-zag median line and evenly spaced, repetitive lateral grooves which extend from the axis at ~70°. No stalks or attachment disks are known¹ for this species.

Strong similarities exist between the Brachina Subgroup fossils and *Pteridinium* found in the late Precambrian sedimentary units of the Nama Group of south-west Africa^{16–18}, the Valdai sediments of the White Sea coast of the USSR^{18–20}, the Carolina slate belt of the southeastern United States²¹, and the Pound Subgroup of the Ediacara Hills, South Australia^{13,22}. The Brachina Subgroup fossils are comparable in size and morphology to those of the Ediacara assemblage. There is no evidence of secondary grooving, as occurs between the anthostoles of the Charniidae, nor of any anchoring device, therefore I provisionally assign the fossils to the genus *Pteridinium* Gürich.

The Ediacara assemblage from the Pound Subgroup (Fig. 2) has been assigned a late Precambrian age^{1,3}, as have similar fossils from various localities around the world. The proposed Ediacaran Period^{5,23}, during which metazoans appear to have become diverse and relatively numerous, has been assigned to the interval 670–550 Myr⁴. In the present study area, the base of the Wilpena Group lies more than 3,000 m stratigraphically below the Ediacara Member of the Pound Subgroup (Fig. 2). The base of the Wonoka Formation (Fig. 2) has been suggested as the lower boundary of the Ediacaran in South Australia⁵. The first early metazoan assemblages were thought to appear at

Fig. 3 Photograph of the frond-like fossils preserved as impressions on thin shale parting in massive sandstone. Specimens *a* and *b* are described in the text. The coin, shown for scale, is 24 mm in diameter.



~640–620 Myr, and became diverse and frequent during the interval ~600–570 Myr. Ediacara assemblages should comprise almost by definition the first occurrences of metazoan body fossils^{1,4}. Radiometric dating suggests an age of 676 ± 204 Myr for the Brachina Subgroup west of the Flinders Ranges²⁴. The discovery of fossils in the Brachina Subgroup that are similar in size to those of the Ediacara assemblage, supports the placement of the lower boundary of the Ediacaran Period at the base of the Wilpena Group⁴. In addition, the fossils are the first unequivocal metazoan remains to be found in the Brachina Subgroup.

The discovery of metazoans in the Brachina Subgroup raises questions concerning radiometric ages and worldwide stratigraphic correlations. This sedimentary unit rests conformably on the Umberatana Group which marks the termination of late Proterozoic glaciation in the Adelaide geosyncline. An event which in several major regions pre-dates the first appearance of Metazoa is the last occurrence of Precambrian tillites¹. Such tillites overlain by Ediacara assemblages are known in Australia, south-west Africa, China, eastern Europe, Newfoundland, North Carolina and Sweden and may provide the potential for global lithological and biostratigraphical correlations. However, the recording of these various first appearances needs to be addressed further in stratigraphic and geochronometric terms.

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Blue light activates electrogenic ion pumping in guard cell protoplasts of *Vicia faba*

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Transmembrane ion exchange and blue-light dependent responses are central processes in plants in general^{1,2} and stomatal guard cells in particular³. Stimuli, including light, cause H⁺ extrusion by stomatal guard cells^{4,5}, K⁺ uptake, osmotic swelling and enlarged stomatal pore apertures, resulting in increased leaf conductance for CO₂ influx and water efflux⁶. The mechanisms underlying ion movement and the specific response of stomata to blue light^{7–9} remain, however, unknown. We have now investigated these phenomena using gas-exchange techniques on leaves and whole-cell patch clamping^{10,11} of protoplasts. Blue light-stimulated stomatal opening in the leaf is correlated with activation of an electrogenic pump in the guard cell plasmalemma which results in hyperpolarizations as large as 45 mV and outward currents as large as 5.5 pA. The pump functions in the absence of K⁺; in the presence of K⁺ an increase in voltage noise upon light-induced hyperpolarization more negative than the K⁺ equilibrium potential implies K⁺ flow through K⁺-selective channels¹². The data are in accord with a chemiosmotic model of stomatal response¹³ whereby active H⁺ extrusion creates an electrochemical gradient for passive ion fluxes. A 25–35-s delay in the onset of hyperpolarization, and its peak several minutes after application of a blue light pulse suggest that blue light functions as an activator, rather than a direct energy source, for ion pumping.

Figure 1 shows a typical change in hydraulic conductivity, commonly referred to as 'stomatal conductance' of a leaf of *Vicia faba* L. (cv. long pod) following application of a transient blue light stimulus under a constant background illumination

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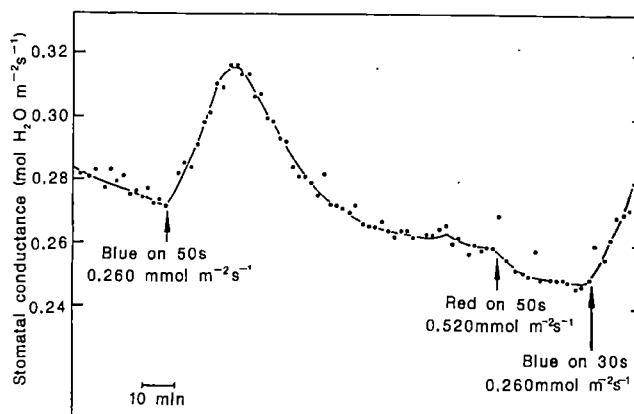


Fig. 1 Transient increase in stomatal conductance in response to a 50-s pulse of $0.260 \text{ mmol m}^{-2} \text{ s}^{-1}$ incident blue light given under $0.525 \text{ mmol m}^{-2} \text{ s}^{-1}$ background red light. Note the absence of any increase in conductance following a 50-s pulse of $0.520 \text{ mmol m}^{-2} \text{ s}^{-1}$ red light applied in the same manner. Background red light was provided by a Sylvania 300W (PAR56/2MEL) lamp used in conjunction with a Kodak 1A red filter (50% cut-off at 645 nm) and a layer of No. 5A Cinemoid. Pulsed light was obtained by filtering light from a GE Gemini 300 projector lamp through a wideband hot mirror (OCLI, Santa Rosa, California) and a Roehm and Haas 2424 blue Plexiglass filter (maximum transmittance at 470 nm, half-bandwidth 100 nm) or a Corning 2-61 red glass filter. Stomatal conductance was measured using a null balance gas-exchange system³⁰. Conditions inside the gas-exchange cuvette were maintained at 23 °C, ambient CO_2 $300\text{--}250 \mu\text{l l}^{-1}$, vapour pressure deficit 0.75–0.95 kPa.

with high fluence rates of red light. As with *Commelina communis*¹⁴, a pulse of blue light results in a transient rise in stomatal conductance to water vapour which peaks ~20 min later. A red light pulse produces no increase in conductance (Fig. 1), demonstrating that the response stimulated by blue light is independent of both photosynthesis and the component of the stomatal response to light which is mediated by the guard cell chloroplasts¹⁵. The response can thus be attributed specifically to the action of the guard cell blue light photoreceptor⁷⁻⁹.

The high specificity provided by this dual-beam protocol^{8,14} was further utilized in an electrophysiological investigation of the membrane properties and ion fluxes underlying the blue light response of stomata. Studies using conventional electrophysiological techniques have shown hyperpolarization in response to white light^{16,17}. We used the whole cell patch-clamp technique^{10,11}, instead of intracellular recording, to ensure an unambiguous characterization of electrical responses of the plasmalemma as opposed to the tonoplast or a series combination of the two membranes¹⁸, thus solving a ubiquitous problem in plant electrophysiology. Patch clamping allowed us to observe either membrane potential (V_m) or membrane current, and to define solutions on both sides of the plasmalemma, so that gradients for passive ion fluxes could be manipulated or eliminated.

Seal formation on single guard cell protoplasts of *V. faba* L. (cv. long pod and grünehangdown) was facilitated by the presence of 1 mM CaCl_2 in the bath solution, by a pipette solution with an osmolality 10% less than that of the bath solution¹⁰, and by the use of Kimax glass (Kimble 34500) pipettes with tip resistances of 10–20 M Ω in symmetrical 150 mM KCl solutions¹⁹. Typical whole-cell membrane resistances were 1–5 G Ω , although resistances as high as 20 G Ω were observed. Immediately after the establishment of the whole-cell configuration, healthy cells exhibited a V_m of –20 to –50 mV which then declined towards 0 mV, presumably as a result of diffusional equilibration between the cell contents and the pipette solution. Cells typically stabilized at resting potentials of –10 to +10 mV.

In symmetrical 50 mM K^+ -glutamate solutions, a 30-s pulse of $0.050 \text{ mmol m}^{-2} \text{ s}^{-1}$ blue light given on a background of $0.500 \text{ mmol m}^{-2} \text{ s}^{-1}$ red light resulted in a transient membrane

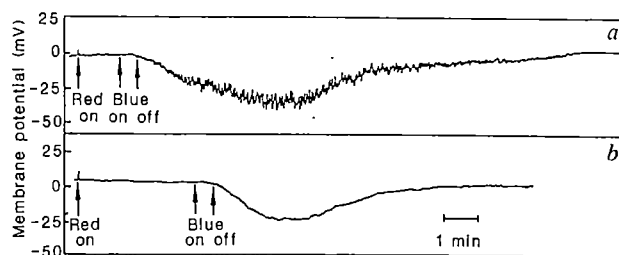


Fig. 2 *a*, Transient membrane hyperpolarization in a guard cell protoplast in whole-cell recording mode, current clamp, in response to a 30-s pulse of $0.050 \text{ mmol m}^{-2} \text{ s}^{-1}$ blue light superimposed on a $0.500 \text{ mmol m}^{-2} \text{ s}^{-1}$ red light background. Incident fluence rates were measured above the air–water interface. Note the increase in voltage noise as the membrane hyperpolarized and the delay before the initiation of the response. Both external and pipette solutions contained 50 mM K^+ -glutamate, 5 mM MgCl_2 , 10 mM HEPES, 3 mM NaOH; internal solution contained in addition 9.2 mM (Na^+)₂-ATP in 20 mM Tris (final concentrations) and 100 μM EGTA. External solution was pH 7.15, 500 mosmol kg^{-1} ; internal solution was pH 7.4, 450 mosmol kg^{-1} . Osmolarities were measured in a vapour pressure osmometer (Wescor 5100c) and adjusted by the addition of D-mannitol. Temperature 22–24 °C. *b*, Membrane hyperpolarization in response to blue light. Solutions were the same as in *a* except that K^+ was replaced by the impermeant cation *n*-methylglucamine in both the external and pipette solutions, and the external solution contained 1 mM CaCl_2 . Note the relative absence of voltage noise. *a* and *b* show no response in dark-adapted cells on an initial exposure to the background red light; occasionally cells showed a transient hyperpolarization when the background light was turned on (not illustrated). The following conditions were identical for *a* and *b*. Background red light was obtained by placing a Kodak No. 29 gelatin (50% cut-off at 590 nm) and a Cinemoid No. 5A filter in the microscope light path (Zeiss 12V, 60-W 1251 bulb). Blue light was obtained by filtering light from a Liesegang Fantimat 150Af projector lamp through a Roehm and Haas 2424 blue Plexiglass filter. Control of membrane current and measurement of membrane potential were performed with an EPC-7 patch clamp (List Electronic) and recorded on a Racal store 4Ds tape recorder and a chart recorder. Protoplasts were obtained by modification of a published procedure³¹. Abaxial epidermal peels from six leaves of 3–6-week-old plants were incubated in 15 ml of 2% Cellulase Onozuka RS (Yakult Honsha Co.) and 0.022% Pectolyase Y-23 (Seishin Pharmaceutical Co.), 0.3 M D-mannitol and 1 mM CaCl_2 for 50–65 min in a shaking water bath (temperature 29–31 °C). Following passage through a 30- μm mesh, the enzyme solution was centrifuged at 125g for 12 min (Sigma 3E centrifuge) and the pellet, containing the guard cell protoplasts, was washed twice (125g for 8 min) in 0.5 M D-mannitol with 1 mM CaCl_2 and stored on ice in the same solution.

hyperpolarization peaking several minutes later (Fig. 2*a*). The characteristics of the hyperpolarization were similar to those of the stomatal response in the whole leaf (compare Figs 1 and 2) but had a faster time course, as might be expected of an early event in the phototransduction process occurring in an isolated system. Patch clamping allowed an accurate resolution of the initial kinetics of the response to blue light, revealing a 25–35-s delay between the onset of the blue light and the beginning of hyperpolarization. An identical delay was observed under longer illuminations with blue light (not illustrated), demonstrating that the onset of hyperpolarization is not correlated with termination of the blue light signal. Similar response kinetics were observed when the major permeant cation, K^+ , was replaced by the impermeant ion *n*-methylglucamine (Fig. 2*b*). In the absence of K^+ , the voltage noise of the response was greatly reduced, suggesting that this noise represents passive flux of K^+ through discrete channels¹² in response to the change in V_m . As in the intact leaf, no such response was observed to a pulse of red light ($n=7$, cf Fig. 3*a*), demonstrating that the responses illustrated in Fig. 2 are mediated by the blue light photosystem.

The blue light response was observed in cells that had resting potentials from –30 to +10 mV and within this range exhibited no apparent voltage dependence ($n=20$, not shown). No response to blue light was observed when ATP was eliminated

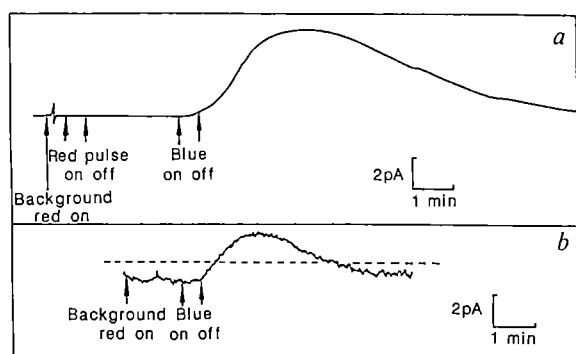


Fig. 3 *a*, Response of whole-cell membrane current to blue light. The membrane potential of the cell was voltage-clamped at $V_m = -4$ mV and the current response was low-pass-filtered at 10 Hz. Smaller current responses were also observed at this voltage (not illustrated). Solutions and illumination protocol were the same as in Fig. 2*b*. Note the absence of a response to a 30-s pulse of $0.050 \text{ mmol m}^{-2} \text{ s}^{-1}$ red light, obtained by transiently increasing the intensity of the microscope light. *b*, Same recording conditions as *a* except that the cell was voltage-clamped at -50 mV and the response was filtered at 60 Hz. Temperature 19°C . The response exceeds the zero current level (dashed line), indicating that blue light activates an outward current rather than an increase in membrane resistance.

from the pipette solution ($n = 7$, not shown). These observations led us to postulate that the hyperpolarization induced by blue light resulted from the activation of an electrogenic plasmamembrane ATPase. To test this hypothesis, the membrane potential was clamped at -50 mV, a potential more negative than the Nernst potential of all significant ionic species present in the external and internal solutions¹¹. The observation under these conditions of a blue light-stimulated outward current exceeding the zero current level (Fig. 3*b*) cannot be explained by passive ion fluxes, providing strong evidence for the operation of an electrogenic pump.

In the responses shown in Fig. 3, blue light induced outward currents as large as 5.5 pA . Given an average guard cell protoplast diameter of $14.1 \text{ } \mu\text{m}$ ($n = 20$), currents as large as $0.88 \text{ } \mu\text{A cm}^{-2}$ can be calculated. Previous observations of light-stimulated release of H^+ from guard cells^{4,5} suggest an electrogenic proton pump as the source of this current. Such a conclusion is further supported by the recent finding²⁰ that when suspensions of *V. faba* guard cell protoplasts illuminated with continuous high-intensity red light are exposed to a 30-s blue light pulse of the same fluence rate as that used in Figs 2 and 3, acidification of the medium occurs at rates comparable to the outward currents reported here.

In many types of plant cells²¹⁻²³, as well as in guard cells, H^+ extrusion appears to be coupled with K^+ uptake. The basis of that coupling is, however, unknown, available data being consistent with either an electrogenic H^+ pump or an antiporter with a stoichiometry for H^+ and K^+ which is not 1:1 (refs 24, 25). Our results showing electrogenic pumping in the absence of K^+ (Figs 2*b*, 3) clearly indicate that the electrogenic pump in guard cells which is activated by blue light is not an obligate K^+ antiporter. We conclude that the most likely mechanism underlying blue light stimulation of stomatal conductance to water vapour in the leaf (Fig. 1) is light activation of a hyperpolarizing proton pump in the guard cell plasmalemma which generates an electrical gradient for passive K^+ flux¹² driving increases in turgor. The data reported here therefore support a chemiosmotic model of stomatal opening¹³.

The results also have specific photobiological implications. The time course of the membrane hyperpolarization, particularly the delay between illumination onset and the initiation of hyperpolarization (Figs 2, 3), and the dependence of the response on ATP, are inconsistent with utilization of blue light as a source of photochemical energy for H^+ extrusion as occurs, for example, in *Halobacterium*²⁶. The response is observed when

guard cells are provided via the pipette solution with 9.2 mM ATP, a greater concentration than is typically measured in plant cells²⁷. Thus, it also seems unlikely that removal of an energy limitation on H^+ extrusion via blue light-induced enhancement of respiration²⁸ is occurring. Rather, blue light seems to function as a regulator of the electrogenic pump. Regulation could occur by a direct modulation of the membrane ATPase or by stimulation of redox reactions²⁹ that result in pump activation. Further application of the whole-cell patch-clamp technique to plant cells should prove valuable in elucidating the processes by which blue light and other environmental stimuli are transduced into cellular responses appropriate for plant function.

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Protection of cottontop tamarins against Epstein-Barr virus-induced malignant lymphoma by a prototype subunit vaccine

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Epstein-Barr (EB) virus is one of the five herpesviruses of man. Strong links between this agent and the chain of events causing two human cancers, endemic Burkitt's lymphoma and undifferentiated nasopharyngeal carcinoma, have long been evident (reviewed in ref. 1). Because of this, and because of the very high incidence of nasopharyngeal carcinoma in certain large populations², it was suggested in 1976 that a vaccine should be developed against EB virus to prevent infection and thereby reduce tumour incidence amongst those at risk³. The virus-determined membrane antigen

(MA) was proposed as immunogen³ because it was known to elicit naturally occurring virus-neutralizing antibodies in man⁴⁻⁷ and because analogous antigens had been shown to act as effective experimental vaccines for preventing the herpesvirus-induced lymphomas of Marek's disease in chickens^{8,9}. Progress has been achieved in defining, quantifying and preparing MA molecules, and in enhancing their immunogenicity¹⁰; a sensitive assay for antibodies to MA has been elaborated¹¹. Here we report that isolated cell membranes expressing MA, or purified MA glycoprotein of relative molecular mass (M_r) 340,000 (gp340), have been used to vaccinate cottontop tamarins (*Saguinus oedipus*), and that animals receiving either preparation were protected against the effects of a 100% tumour-inducing challenge dose of EB virus.

The cottontop tamarin is the only species in which experimental EB virus infection regularly causes lesions¹² and recent investigations have established that these are true, clonally derived, malignant lymphomas¹³. Young adult tamarins from a successful breeding colony¹⁴⁻¹⁶ were used in the present studies.

For the membrane experiments, B95-9 cells grown as reported earlier¹⁷ and showing maximum expression of MA after treatment with tetradecanoyl phorbol acetate¹⁸, were disrupted and the plasma membranes collected by sucrose density gradient centrifugation¹⁹. The resulting material contained only trace amounts of infectious virus when titrated in fetal cord blood lymphocytes²⁰, several logs less than is necessary for infection. The purified membranes were injected intraperitoneally (i.p.) eight times into two tamarins at fortnightly intervals, each inoculation containing on average 2,500 units of MA gp340 as assessed by radioimmunoassay (RIA)¹⁷. Serological responses were measured by indirect immunofluorescence, a highly sensitive enzyme-linked immunosorbent assay (ELISA)¹¹, and standard virus neutralization tests which assess the ability of a given serum to prevent transformation by the virus of fetal cord blood lymphocytes^{7,20}; all neutralizations were repeated (up to three times) and included control sera of known titre, so as to take account of the inherent variations in sensitivity shown by cells from different cord bloods. Specific antibodies were rapidly

Table 1 EB virus-induced lymphomas in unprotected cottontop tamarins

Expt no.	Lymphocyte-transforming units of virus injected	Injection route	Animals with gross multiple lesions
1	$10^{5.3}$	i.v.; i.p.; i.m.	2/2
2	$10^{5.3}$	i.p.; i.m.	2/2
3	$10^{5.3}$	i.p.; i.m.	4/4
4	$10^{5.3}$	i.p.; i.m.	2/2
5	$10^{5.3}$	i.p.; i.m.	4/4
6	$10^{5.3}$	i.p.; i.m.	2/2
7	$10^{5.3}$	i.p.; i.m.	2/2
8	$10^{5.3}$	i.p.; i.m.	2/2
9	$10^{5.3}$	i.p.; i.m.	4/4

induced, eventually reached high titre, and were then powerfully virus-neutralizing; because the neutralization tests were all repeated more than once, neutralizing titres were recorded as being greater than the minimum obtained in each set of tests. The tamarins were considered to have been satisfactorily immunized when their levels of neutralizing antibody equalled or exceeded the highest titres found in sera from naturally infected human subjects.

The immunized animals were challenged with $10^{5.3}$ lymphocyte-transforming units of virus²⁰, a dose checked in advance for its ability to produce gross, multiple, malignant tumours within 21 days¹³ in 100% of unprotected tamarins (Table 1). The tumours presented as masses (not infiltrates) in spleen, liver, kidneys, gut wall and adrenals, and in numerous lymph nodes, causing enlargement from the normal 1-2-mm diameter up to 15-20 mm (ref. 13). Use of a 100% lymphoma-inducing challenge dose of virus was considered important for biological tests in a species where only small numbers of animals are available and inbred strains are unknown, but to ensure exclusion of equivocal results, two additional normal control

Table 2 Protection of cottontop tamarins against EB virus-induced lymphomas by vaccination with B95-8 cell membranes

Tamarin	Antibody to B95-8 cell surface (immunofluorescence)	ELISA antibody titre	Virus neutralizing antibody	Lesions after $10^{5.3}$ units of virus i.p.; i.m.
	+++	1:800	Strong*	None
	+++	1:2,000	Strong*	None
	-	0	None	Gross multiple
	-	0	None	Gross multiple

* 1 ml serum neutralized >100,000 lymphocyte-transforming units of virus (from lowest value in repeated tests).

Table 3 Protection of cottontop tamarins against EB virus-induced lymphoma by vaccination with MA gp340 purified from B95-8 cell membranes by M_r -based method²¹

Expt	Tamarin	Antibody to MA (immunofluorescence)	ELISA antibody titre	Virus neutralizing antibody	Lesions after $10^{5.3}$ units of virus i.p.; i.m.
a, Pilot	Vaccinated	+++	1:1,000	Strong†	None
	Vaccinated	+	1:100	None	Gross multiple
	Vaccinated	+	<1:10	Slight	Gross multiple
	Vaccinated	+	*	None	Gross multiple
b, Confirmatory	Vaccinated	+++	1:800	Strong‡	2 Small, transient
	Vaccinated	+++	1:1,000	Strong§	Single, transient
	Control	-	0	None	Gross multiple
	Control	-	0	None	Gross multiple

* Serum sample haemolysed; not testable.

† 1 ml serum neutralized >10,000 lymphocyte-transforming units of virus.

‡ 1 ml serum neutralized 100,000 lymphocyte-transforming units of virus.

§ 1 ml serum neutralized >100,000 lymphocyte-transforming units of virus. All results are from the lowest value in repeated tests.

animals were also given the challenge material at the same time. The immunized tamarins remained entirely free of clinically detectable lesions whereas the normal control animals developed the expected gross multiple tumours within 2–3 weeks (Table 2).

Because it was known that purified MA gp340 prepared by a M_r -based method which conserved immunogenicity²¹ induced virus-neutralizing antibodies in various species when injected after incorporation in liposomes^{22,23}, we investigated the protective effects of these antibodies in tamarins, and the levels required for protection. In a pilot experiment, four animals were selected in whom differing amounts of neutralizing antibody were present after i.p. immunization with liposomal gp340, and all were then challenged with the 100% lymphomagenic dose of virus (Table 1). Table 3a shows that only the animal whose serum was strongly neutralizing was completely protected, whereas the other animals with much lower, or undetectable, levels of neutralizing antibody were not protected at all.

To confirm this result, two further tamarins were given gp340 in liposomes 17 times i.p. at fortnightly intervals, each dose containing on average 2,250 RIA units¹⁷. Antibodies were monitored as before and when these had reached high levels (Table 3b) the vaccinated animals were challenged with the 100% pathogenic dose of EB virus (Table 1), along with 2 normal control animals. The unprotected animals developed gross multiple tumours within 2–3 weeks whilst the vaccinated tamarins remained free of tumours (Table 3b); one protected animal exhibited minor transient swelling of a single inguinal lymph node draining the site of intramuscular (i.m.) injection in the thigh muscle, and the other developed similar inguinal node enlargement to 5 mm which rapidly regressed, accompanied by minor transient enlargement of a single mesenteric node.

These experiments demonstrate that gp340 prepared by the M_r -based method²¹ can be used as an efficient prototype subunit vaccine to protect susceptible experimental animals against tumour induction by a massive challenge dose of EB virus. The protected tamarins have remained well for more than 9 months and quite recent experiments with larger doses of gp340 have shown rapid induction of high-titre antibodies and followed the kinetics of their development²⁴. Investigation of the overall structure of the gp340 molecule has already indicated that more than 50% of its total mass is carbohydrate and that it is the protein component which is antigenic²⁵. The region of EB virus DNA carrying the gene for MA has been identified²⁶ and the nucleotide sequence of this gene is known²⁷; MA production by expression in pro- and eukaryotic cells therefore seems possible, and synthetic gp340 peptides could also be considered as immunogens, perhaps in combination with powerful new adjuvants²⁸. It should similarly be feasible to incorporate the MA gene into the genome of vaccinia virus to permit direct expression during vaccination with this agent^{29–31}. The present results establish a strong case for determined work in these directions to produce MA gp340 in a form suitable for use as a vaccine in humans.

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Specific release of proteoglycans from human natural killer cells during target lysis

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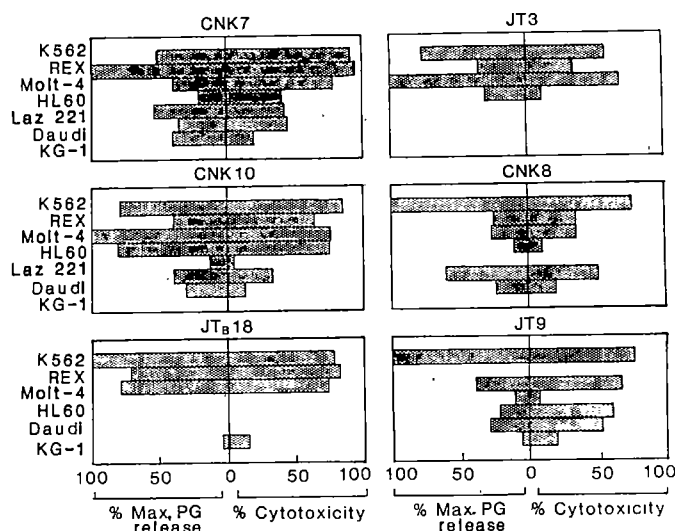
Natural killer (NK) activity is mediated by a small population of peripheral blood cells that exhibit the homogeneous morphology of large granular lymphocytes (LGL)^{1–4}. In recent studies, human NK cell clones^{5,6} have been shown to contain a 200,000- M_r (relative molecular mass) protease-resistant chondroitin sulphate A proteoglycan, which has been localized to the secretory granule by X-ray dispersive analysis and by its resistance to cleavage by extracellular addition of chondroitinase AC or ABC (ref. 7). In the present study, we have used six different human NK cell clones to demonstrate that release of ³⁵S-proteoglycan correlates closely with cytolytic activity against various NK cell targets. When NK activity is blocked by monoclonal antibodies at either the effector cell level (LFA-1)⁸ or at the target cell level (TNK_{TAR})⁹, there is a concomitant decrease in exocytosis of proteoglycan. Monoclonal antibodies directed against recognition structures, for example anti-NK1a and anti-T3 (ref. 10), function as soluble stimuli, capable of initiating the release of ³⁵S-proteoglycan. Taken together, these results provide strong evidence for the stimulus-specific release of chondroitin sulphate A proteoglycans from NK cells when the cytolytic process is activated.

To determine whether chondroitin sulphate A proteoglycan was consistently released in the cytolytic mechanism of NK cells, we used six different human NK cell clones selected because of their ability to maintain stable cytolytic activity against a series of allogeneic target cells without previous immunization^{5,11}. Phenotypic analysis of these clones indicated that each expressed NKH1 antigen, a surface structure of 200,000- M_r associated with all NK cells in peripheral blood¹², and the T11/E rosette receptor. Clones JT3, CNK7 and CNK10 also expressed NKH2 antigen¹², while clone JT_B18 was NKH2 negative. Together, these four clones, which do not express the T3 antigen or T-cell receptor-like structures, represent the most common phenotypes of circulating NK cells^{2–4,12}. Clones JT9 and CNK8 bear NK1a, a T-cell receptor-like structure that is linked to surface T3 (ref. 10). Moreover, both clones are specific

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Fig. 1 Comparison of natural cytotoxicity and proteoglycan release from NK clones (CNK7, JT3, CNK10, CNK8, JT_B18 and JT9) interacting with seven target cell lines (K562, REX, Molt-4, HL60, Laz 221, Daudi and KG-1).

Methods. Cytotoxicity experiments were performed in triplicate using V-bottomed microtitre plates. Medium for cytotoxicity assays was RPMI 1640 containing 5% pooled human AB serum and 1% penicillin-streptomycin. Assays were performed at an effector/target cell ratio of 10:1 using 5,000 ⁵¹Cr labelled target cells per well. Cytotoxicity was determined after 4 h of incubation at 37 °C by measuring release of ⁵¹Cr into the cell supernatant. Specific cytotoxicity was calculated according to a standard method¹¹. Simultaneously, duplicate cultures of the same cloned NK cells were used in proteoglycan release experiments. For the latter studies, NK cells were incubated with 100 µCi of ³⁵S-sulphate ml⁻¹ at concentrations of 2 × 10⁶ cells ml⁻¹ for 24 h at 37 °C. After ³⁵S-sulphate labelling, the cloned NK cells were centrifuged and washed twice to remove unincorporated ³⁵S-sulphate. ³⁵S-labelled NK cells (25,000) were incubated with 50,000 target cells (effector/target ratio of 0.5:1) in quadruplicate in V-bottomed microtitre plates for 4 h at 37 °C. Supernatants (150 µl) from two of the four wells were combined, resulting in duplicate 300-µl samples for analyses. Each 300-µl supernatant sample was applied, together with 25 µl of chondroitin sulphate carrier and 250 µl of 8 M guanidin hydrochloride (GnHCl), to separate PD-10 columns (1.5 × 5.5 cm) which were equilibrated and eluted under dissociative conditions of 4 M GnHCl, 0.1 M Tris-HCl, 0.1 M Na₂SO₄, pH 7.0. Eight 0.5-ml fractions were collected and after the addition of 0.5 ml ethanol and 12.5 ml Hydrofluor to each fraction, ³⁵S-radioactivity was quantitated using a β-scintillation counter. The total proteoglycan content (TPG) was determined by addition of 1% Nonidet p-40. Spontaneous release (SR) was obtained from labelled effector cells incubated with media in the absence of targets (8.5 ± 4.5%, mean ± s.d.). Stimulus-specific per cent proteoglycan (PG) release was calculated according to the following formula, in which experimental release (ER) is release occurring in the presence of a target cell: % specific PG release = (ER - SR) / (TPG - SR) × 100. To compare the proteoglycan release of various NK clones in experiments done on different days, we expressed the release of labelled proteoglycan as a percentage of the maximal proteoglycan release obtained with the optimal NK/target combination in a given experiment which included K562 three times, Molt-4 twice, and REX once. In the experiments shown, maximal release represented 46% of total proteoglycan content for the combination CNK7/REX, 36% for CNK10/Molt-4, 48% for JT_B18/K562, 12% for JT3/Molt-4, 52% for CNK8/K562 and 69% for JT9/K562.



for a 140,000-*M_r* surface antigen that is linked to cell activation and is identified by two monoclonal antibodies, anti-TNK_{TAR} (ref. 9) and 4F2 (refs 13, 14), which have been shown to block target cell killing by NK clones expressing the NKTa clonotype.

Cytotoxicity was determined by measuring ⁵¹Cr release into the cell supernatant after incubation of unlabelled effector cells with ⁵¹Cr-labelled target cells at a ratio of 10:1 at 37 °C for 4 h¹¹. Release of ³⁵S-proteoglycan into the cell supernatant was determined during incubation of ³⁵S-labelled effector cells with unlabelled target cells at a ratio of 0.5:1 for 4 h at 37 °C. To compare proteoglycan release by different targets with toxicity against these cells, the maximum release of ³⁵S-proteoglycan from each NK clone in a given experiment was considered to represent 100% release and all other release values were expressed as a per cent of maximal release. As shown in Fig. 1, specific cytotoxicity closely paralleled proteoglycan release; this was found for T3-positive (T3⁺) as well as for T3-negative (T3⁻) NK clones. Figure 2 summarizes the results of 34 experiments using six different NK clones and seven different targets; there was a linear relationship between percentage cytotoxicity and percentage maximal proteoglycan release (correlation coefficient 0.81).

The specific exocytosis of ³⁵S-proteoglycan was also evaluated in conditions in which NK cytotoxicity was inhibited at either the effector cell or target cell level. Monoclonal antibody 2F12, which is specific for LFA-1 antigen^{15,16} and is known to block cytotoxicity of NK clones at the effector cell level⁸, inhibited cytotoxicity of both T3⁺ and T3⁻ NK clones, with the greatest inhibition occurring with Molt-4, CEM and REX targets (Fig. 3). Reductions in exocytosis of ³⁵S-proteoglycans closely paralleled the inhibition of cytotoxicity. The monoclonal antibody anti-TNK_{TAR} inhibited the NK cytotoxicity at the target cell level of the T3⁺ clones JT9 and CNK8, both of which express the NKTa clonotype (Fig. 3). In these experiments, specific release of proteoglycans from JT9 and CNK8 was also markedly reduced, whereas there was no change in either cytolytic function or release of proteoglycans from clones JT_B18 and JT3. The correlation coefficient between percentage maximal

proteoglycan release and percentage cytotoxicity for all experiments shown in Fig. 3 was calculated to be 0.92.

Release of proteoglycans from NK cell clones was also evaluated under conditions known to trigger activation of NK cells without the presence of target cells. The results (Table 1) demonstrate that binding of monoclonal anti-T3 and anti-NKTA antibodies was effective in triggering release of ³⁵S-proteoglycans from NK cell clones JT9 and CNK8 but not from JT_B18 and JT3. The former two clones, but not the latter two, are known to use a T-cell receptor-like complex in expressing their cytolytic function¹⁷. Monoclonal anti-T6, which was used as a negative control antibody, did not initiate any exocytosis of proteoglycan. In contrast, the lectins concanavalin A (Con A) and Phytohaemagglutinin (PHA) were able to trigger release of proteoglycan from all NK clones regardless of differences in cell surface phenotype, with PHA being more effective than Con A. Control experiments using these monoclonal antibodies showed that the viability of the cells before and after triggering by trypan blue exclusion was never less than 95% and that the lactate dehydrogenase release into the cell supernatant was never greater than 5%.

The data presented here provide strong evidence that proteoglycan exocytosis occurs concomitantly with stimulus-specific

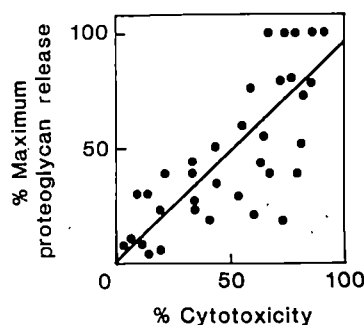


Fig. 2 Correlation of per cent specific cytotoxicity with per cent maximal proteoglycan release for 34 different experiments. $r = 0.81$.

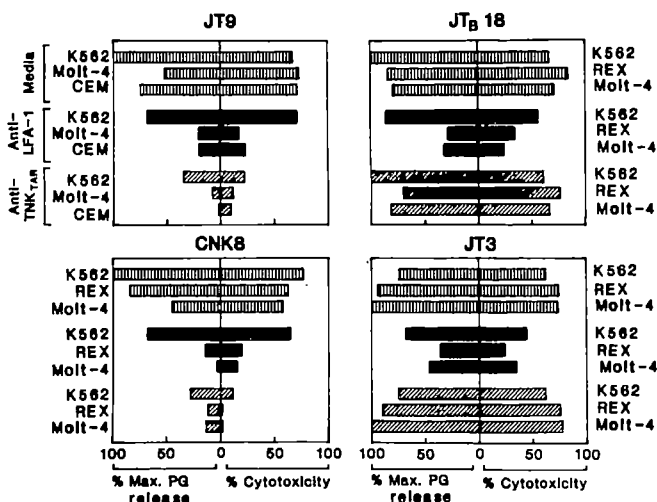


Fig. 3 Natural cytotoxicity and proteoglycan release are inhibited by anti-LFA-1 and anti-TNK_{TAR} antibodies, which exert their blocking activity through epitopes on the NK and target cells, respectively. Either effector cells or target cells were incubated with media (▨) or monoclonal antibody anti-LFA-1 (■) or anti-TNK_{TAR} (▤) for 30 min at room temperature before the assay, and both specific cytotoxicity and per cent maximal proteoglycan release were measured as described in Fig. 1 legend, except that cytotoxicity experiments were performed using an effector/target cell ratio of 5:1. Maximal proteoglycan release was 65% of total proteoglycan content for the combination JT9/K562, 46% for CNK8/K562, 46% for JTB18/K562 and 13% for JT3/Molt-4.

activation of the cytolytic capacity of NK cells. In a companion study of cloned NK cells, ³⁵S-proteoglycan was demonstrated to be localized in intracellular granules by X-ray dispersive analysis and was shown to be a protease-resistant chondroitin sulphate A proteoglycan⁷. By using a panel of six human NK cell clones and a variety of different target cells, we now also demonstrate a linear correlation between specific cytotoxicity and release of proteoglycan (Figs 1, 2), indicating that this secretory granule constituent is specifically released during the NK cytolytic process. This conclusion is further supported by the demonstration that blocking of NK activity by monoclonal antibodies anti-LFA-1 and anti-TNK_{TAR}, which inhibit cytotoxicity at the effector cell and target cell level, respectively, also concurrently inhibit the release of intracellular proteoglycans (Fig. 3). In addition, triggering of NK clones with soluble agonists through specific receptors or through binding of Con A and PHA similarly resulted in the release of radiolabelled proteoglycans (Table 1).

Protease-resistant chondroitin sulphate A proteoglycan may function as a carrier for cytolytic proteins or may protect the NK cell from injury by the cytotoxic molecules which are released during the lethal reaction^{18,19}. The results presented define the secretory nature of the NK cellular response in terms of a secretory granule marker, intracellular proteoglycan, and are compatible with the stimulus-specific nature of target cell cytotoxicity.

Table 1 ³⁵S-Proteoglycan release by NK clones activated with soluble stimuli

NK clone	Anti-T6	Anti-T3	Anti-NKTA	Con A	PHA
JTB18	0	0	0	17	26
JT3	0	0	0	7	63
JT9	0	25	28	20	59
CNK8	0	46	40	14	29

Values are per cent total proteoglycan release from various cloned NK cells incubated for 4 h with monoclonal antibodies anti-T6 (control), anti-T3 and anti-NKTA, or the lectins PHA (20 µg ml⁻¹) and Con A (20 µg ml⁻¹). Proteoglycan release was determined as described in Fig. 1 legend.

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A *trans*-acting class II regulatory gene unlinked to the MHC controls expression of HLA class II genes

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Class II (or Ia) antigens are highly polymorphic surface molecules which are essential for the cellular interactions involved in the immune response. In man, these antigens are encoded by a complex multigene family which is located in the major histocompatibility complex (MHC) and which comprises up to 12 distinct α - and β -chain genes, coding for the HLA-DR, -DQ and -DP antigens^{1,2}. One form of congenital severe combined immunodeficiency (SCID) in man, which is generally lethal, is characterized by an absence of HLA-DR histocompatibility antigens on peripheral blood lymphocytes (HLA class II-deficient SCID). In these patients, as reported here, we have observed an absence of messenger RNA for the α - and β -chains of HLA-DR, -DQ and -DP, indicating a global defect in the expression of all class II genes. Moreover, the lack of expression of HLA class II mRNAs could not be corrected by γ -interferon, an inducer of class II gene expression in normal cells. Family studies have established that the genetic defect does not segregate with the MHC. We conclude, therefore, that the expression of the entire family of class II genes is normally controlled by a *trans*-acting class II regulatory gene which is unlinked to the MHC and which is affected in the patients. This gene controls a function or a product necessary for the action of γ -interferon on class II genes.

The expression of the entire class II multigene family is restricted to a limited number of cell types, is strictly regulated developmentally, and is coordinately induced by soluble mediators (such as γ -interferon). Cells from class II-deficient SCID cases could thus provide a model for the study of the regulation of this gene family. In these patients, the absence of expression of class II antigens on lymphocytes and macrophages³ explains the immunological unresponsiveness

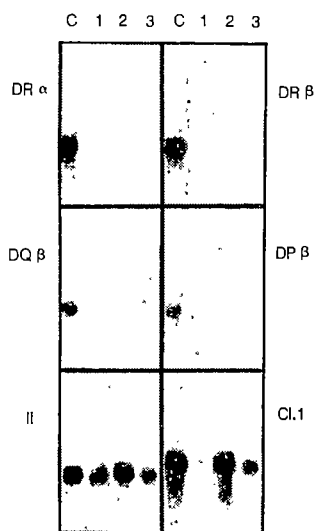


Fig. 1 Northern blot analysis of mRNAs from PHA-stimulated blasts of HLA class II-deficient SCID patients (lanes 1-3) and of a normal healthy donor (C).

Methods. RNA preparation and Northern blot analysis were performed as reported elsewhere²⁰. Briefly, total RNA from PHA-blasts was prepared by the guanidinium thiocyanate-caesium chloride procedure, denatured in glyoxal and electrophoresed in a 1.8% agarose gel (20 g per slot). RNA was transferred to diazobenzylmethyl paper, and the filters were hybridized in 50% formamide, 0.75 M NaCl, 10% dextran sulphate at 42°C, with nick-translated ³²P-cDNA probes (specific activity 1.5–3 × 10⁸ c.p.m. g⁻¹). The final washes were done under stringent conditions: 0.1 × SSC buffer, 68°C, twice for 30 min each before autoradiography. The following ³²P-labelled probes were used: DRα⁵, DRβ and DQβ⁶, DPβ⁷, invariant chain (II)⁸, and class I heavy-chain pHLA 44/2 (CI.1; ref. 21). Some blots were dehybridized in 99% formamide, 5 mM Tris-HCl pH 7.5, 1 mM EDTA, 65°C, three times for 10 min each, and rehybridized. The specific probe used for each of the six experiments is indicated. The films were exposed for 4 days; prolonged exposure (17 days) gave identical results (data not shown). RNAs from fresh peripheral blood lymphocytes from one patient gave identical results.

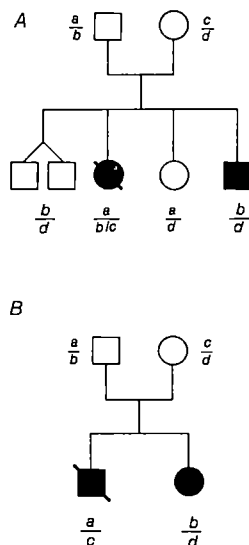
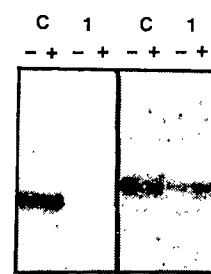


Fig. 2 Segregation of HLA haplotypes in two families. Open symbols, healthy individuals; solid symbols, HLA class II-deficient SCID patient; ●, ■, deceased individuals. **A**, The healthy twins and their affected brother are haplo-identical with respect to HLA. Haplotypes: *a* = A2 B12; *b* = Aw32 B40; *c* = A1 B21; *d* = Aw32 B8. **B**, The two immunodeficient siblings do not have any HLA haplotypes in common. Haplotypes: *a* = A26 B35 DR2; *b* = A19.2 B35 DR3; *c* = A19.2 B7 DR1; *d* = A3 B7.

Fig. 3 Northern blot analysis of lymphocyte mRNA from HLA class II-deficient SCID patients (lanes labelled 1) and a healthy donor (C) following *in vitro* stimulation with γ -interferon. The probes used were: **A**, DRβ; **B**, CI.1.



Methods. Blood lymphocytes from patient 1 and from a healthy donor were cultured for 48 h in RPMI 1640 medium, 10% fetal calf serum, in the presence of recombinant interferon (500 U ml⁻¹), provided by Biogen SA. RNA preparation and Northern blot analysis were performed as described in Fig. 1 legend. The blot in **A** was first hybridized with DRβ cDNA⁶; the final washes were performed in 0.5 × SSC buffer at 65°C. (In these conditions, the DRβ ³²P-labelled cDNA also hybridizes to DQβ and DPβ mRNAs.) This blot was dehybridized and rehybridized with an HLA class I ³²P-cDNA probe, CI.1 (ref. 20).

and the dramatic clinical course, which results frequently in death. In 12 patients with HLA class II-deficient SCID, we had observed an absence of HLA-DR molecules on peripheral blood lymphocytes, even after stimulation with phytohaemagglutinin (PHA)⁴. Other cells of the body that are normally HLA-DR-positive were also totally negative. In three of the patients, we have studied the expression of the different genes of the entire *HLA-D* region, using specific HLA class II α- and β-chain complementary DNA probes, described previously⁵⁻⁷.

Messenger RNA for HLA-DR, -DC and -SB antigens (α- or β-chains) could be easily detected in blood lymphocytes from normal individuals. In contrast, no class II mRNA was found in samples of stimulated lymphocytes from patients with HLA class II-deficient SCID (Fig. 1), even after prolonged exposure of the autoradiographs. This block in mRNA expression concerns the three subregions *HLA-DR*, *-DQ* and *-DP*. However, a normal amount of mRNA for the HLA-DR-associated invariant chain (II)⁸ was observed. This difference is of interest in view of the general coupling of expression of the *Ii* gene with that of HLA class II genes both in specific tissues and following induction with γ -interferon^{9,10}. RNA for HLA class I antigens either was present in a normal amount or was somewhat decreased.

SCID seems to be inherited as an autosomal recessive trait, with a high rate of consanguinity and a high frequency of families with two or more affected children of both sexes^{3,4}. As the defect of class II expression concerns not only bone marrow-derived cells, but also all cells that can express class II antigens in normal individuals, we are dealing with a germline defect which consists of a global inability to express HLA class II genes in the form of mRNA. The defect is not only global in terms of all the cell types involved, but it is also multigenic and involves each of the multiple α- and β-chain genes within the *HLA-D* region. Individual mutations in each of these genes are unlikely and massive deletions have been ruled out as a possible explanation by the finding of a normal restriction pattern for *DR* and *DC* genes by Southern blot hybridization¹¹. Cells from one class II-deficient SCID patient were found to revert to a HLA-DR-positive phenotype after 3–5 weeks in Epstein-Barr virus-induced culture⁴. Another argument for the structural integrity of class II genes in these patients comes from an analysis of the family pedigree, including the HLA typing of the parents and relatives. Figure 2A shows a family in which two children are haplo-identical for the MHC, yet one is healthy and the other is affected. Figure 2B illustrates another family in which two siblings are non-identical with respect to the MHC, yet both are affected. Therefore, it can be concluded that the genetic defect which results in a block of class II gene expression does not segregate with the MHC. To our knowledge, no animal model with such a mutation has yet been described.

γ -Interferon is known to stimulate the expression of class II genes, even in cells that do not normally express them^{9,10}. However, the lack of expression of class II genes in HLA class II-deficient SCID could not be corrected by γ -interferon. Figure 3 shows the result of an analysis of RNA from lymphocytes

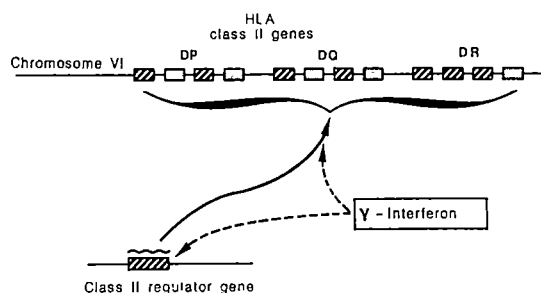


Fig. 4 A class II regulatory gene maps outside the major histocompatibility complex. This gene, or its product, is a potential target for γ -interferon (see text).

from a patient and from a normal control, following incubation in the presence and absence of γ -interferon. The unresponsiveness to interferon of the class II genes contrasts with the response of class I genes (Fig. 3), indicating that the cells from the immunodeficient individuals possess the γ -interferon receptor and are capable of responding.

In these SCID patients, therefore, the class II structural genes are intact and the defect in their expression is caused by a mutation(s) in a gene located outside the MHC. Thus, a trans-acting gene unlinked to the MHC is involved in the normal regulation of the expression of the class II multigene family (Fig. 4). The exact mechanism of this regulation could be transcriptional or post-transcriptional. In this regard, it is of interest that all class II genes of man and mouse (α - and β -genes) share two short sequences 5' upstream of the genes, referred to as 'class II boxes'^{12,13}. From the unresponsiveness of class II-deficient SCID cases to γ -interferon, it is also evident that the induction of class II gene expression observed with γ -interferon in normal cells requires the normal functioning of the class II regulatory gene. The target for the effect of γ -interferon could be the regulatory gene itself, or its product. The expression of the invariant chain gene, although induced by γ -interferon, is probably not under the control of the same regulatory gene.

It is possible that the DR-negative variants^{14,15} in which we had also observed an absence of class II mRNA with the presence of mRNA for class I and invariant chains^{16,17}, represent an analogous situation. Interestingly, fusion with a DR-positive B cell has resulted in the re-expression of the DR antigen of the variant type¹⁸. It has also been shown that fusion of a human DR-positive line with several mouse Ia-negative lines resulted in a DR-negative phenotype, even when the human MHC was still present in the hybrid cells¹⁹. The latter observation is also compatible with the existence of a class II regulatory gene, which would be lost in most of these fusions. Finally, the model presented in Fig. 4 can also accommodate a class II regulation of a negative kind (analogous to the repressors of prokaryotes) in which induction of class II genes would normally result from the inhibition of a negative signal.

The study of HLA class II-deficient patients has thus provided evidence for a novel gene controlling the expression of Ia antigens. One can anticipate that, in the future, this gene may be isolated and transfected into bone marrow stem cells of immunodeficient patients in order to achieve somatic correction of the disease.

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A role for N_i in the hormonal stimulation of adenylate cyclase

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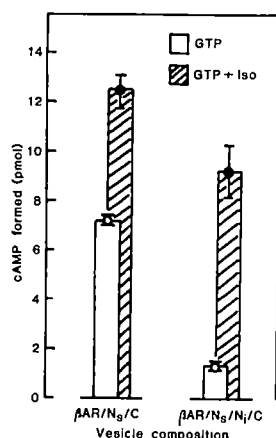
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The best understood system for transduction of extracellular messages into intracellular signals is the hormone receptor-coupled adenylate cyclase. In such systems receptors are functionally coupled to the enzyme by two special proteins, termed the stimulatory and inhibitory guanine nucleotide regulatory proteins (N_s and N_i , respectively). These proteins, thought to mediate, respectively, stimulatory and inhibitory influences on the adenylate cyclase, are members of a larger class of heterotrimeric guanine nucleotide regulatory proteins involved in membrane signal transduction¹⁻⁵. We have studied the interactions of the various components of the adenylate cyclase system by co-reconstituting pure β -adrenergic receptors, pure N_s and N_i , and functionally resolved preparations of the catalyst in phospholipid vesicles. In the absence of N_i , β -adrenergic receptor/ N_s -mediated catecholamine stimulation of the enzyme is relatively modest (~1.3-fold). Surprisingly, however, when N_i is also present, stimulation increases dramatically (up to 7-8-fold) because of a greater suppression of basal relative to agonist-stimulated enzyme activity. Thus, N_i may actually be required for maximal agonist stimulation as well as for inhibition of the adenylate cyclase.

It is believed that hormonal stimulation of the adenylate cyclase (C) system is a simple vectorial signal transduction process whereby the receptor first facilitates the activation of N_s by GTP and the GTP/ N_s complex then activates adenylate cyclase. The stimulation process is terminated by the GTPase activity of N_s . Recently, we reported that the co-insertion into phospholipid vesicles of the pure guinea pig lung β -adrenergic receptor (β AR), with the pure human erythrocyte N_i protein and a resolved bovine caudate adenylate cyclase preparation (that is, resolved from β AR and N_s by Sepharose-6B chromatography) indeed results in the establishment of a modest responsiveness of adenylate cyclase activity to isoprenaline⁶. The results of Fig. 1 confirm this observation. For the conditions shown in Fig. 1 the maximal stimulation by isoprenaline never exceeds two fold and in some cases is nearly non-existent. The average (fold) stimulation by isoprenaline in 11 such experiments is 1.32 ± 0.07 (mean \pm s.e.m.). It seems likely that the modest effect of isoprenaline in these three component systems reflects, at least in part, the effective activation of N_i molecules by GTP alone, thus resulting in a significant basal level of cyclic AMP production.

Fig. 1 The effect of N_i on β -adrenergic-mediated adenylate cyclase activity in phospholipid vesicles. The data are presented as the means of triplicate determinations, with the ranges denoted by error bars.

Methods. Resolved bovine caudate adenylate cyclase (C) preparations (150 μ l; see below), 1.14 μ g of human erythrocyte N_s (≈ 12 pmol), 12 pmol of guinea pig lung β -adrenergic receptor (β AR) and 1.8 μ g of human erythrocyte N_i (≈ 19 mol) were inserted into phospholipid vesicles as follows. Soybean phosphatidylcholine (0.1 ml of a 17 mg ml⁻¹ sonicated solution; Sigma) was incubated with the adenylate cyclase preparation (0.15 ml), 0.08–0.12 ml of 100 mM NaCl, 10 mM Tris-HCl pH 7.4 and 25 μ l of octyl glucoside (17%) for 20–30 min on ice. At this point the nucleotide regulatory proteins (30 μ l of N_s , 36 μ l of N_i) and the receptor [40 μ l of β AR which had been preincubated with 10 μ l of alprenolol (5 μ M) and 10 μ l of bovine serum albumin (BSA, 50 mg ml⁻¹)] were added to the lipid solutions, which had been incubated for ~1–2 min at 4 °C. Rhodopsin (195 pmol) was also included in the incubations containing N_i as we had found previously that rhodopsin promotes the maximal activation of this regulatory protein¹¹. However, under the conditions described for these experiments, N_i alone is effectively activated and essentially identical results are obtained in the absence of rhodopsin. These incubation mixtures were then immediately applied (at 4 °C) to Extracti-gel columns (1 ml of gel) pretreated with 4 vol. of 100 mM NaCl, 10 mM Tris-HCl pH 7.4 containing 2 mg ml⁻¹ of BSA, then equilibrated with 100 mM NaCl, 10 mM Tris-HCl pH 7.4. The protein-vesicles, in the column eluate, were then pelleted using polyethylene glycol essentially as described previously¹¹. The resultant protein-lipid pellets were resuspended in 75 mM Tris-HCl pH 7.8, 1 mM dithiothreitol (DTT, final volume 0.35 ml) and 20 μ l of the vesicles were assayed for adenylate cyclase activity as described below. The β -adrenergic receptor was purified ($\geq 95\%$) from guinea pig lung membranes as described previously¹⁶. The pure receptor preparations were concentrated to ~300 pmol ml⁻¹ using an Amicon concentration cell with a YM 30 membrane, and then stored at -90 °C in 50 mM Tris-HCl, 50 mM NaCl, 0.2% digitonin pH 7.4. N_s and N_i were purified ($>90\%$) as described by Codina *et al.*¹² and stored at -90 °C in 10 mM HEPES pH 8.0, 1 mM EDTA, 20 mM β -mercaptoethanol, 30% ethylene glycol, 150 mM NaCl, 50 μ g ml⁻¹ BSA and in 1.65–10% Lubrol PX. The catalytic moiety (C) of adenylate cyclase was solubilized from bovine caudate nucleus with sodium cholate and isolated from the other components of the system by Sepharose-6B chromatography as described elsewhere^{6,17}. These preparations were stored in 50 mM Tris-HCl, 200 mM sucrose, 1 mM DTT, 15 mM MgCl₂, 3.5 mg ml⁻¹ crude soybean phosphatidylcholine and 0.6% sodium cholate, pH 7.6. The concentrations of β AR are expressed in terms of [¹²⁵I]-iodocyanopindolol binding activity. The amounts of N_s and N_i are expressed in terms of total protein as described previously¹²; the molar concentrations of these proteins were determined assuming a relative molecular mass of 95,000 (ref. 12). The adenylate cyclase assays were performed in a total volume of 0.1 ml containing 50 mM Na-HEPES pH 8.0, 1 mM EDTA, 0.25 mM ATP, 3 mM phosphoenolpyruvate, 10 μ g ml⁻¹ pyruvate kinase, 0.1 mg ml⁻¹ BSA, 0.1 mM cyclic AMP (cAMP), 10 μ g ml⁻¹ myokinase, 2 μ Ci of [³²P]ATP and 2 mM MgCl₂. Isoprenaline (Iso) = 0.1 mM; GTP = 10 μ M.



The construction of a vesicle system comprised of pure β AR, pure N_s , resolved adenylate cyclase and pure human erythrocyte N_i results in three striking effects (Fig. 1): (1) the absolute levels of adenylate cyclase activity are reduced (relative to β AR/ N_s /C vesicles) both when assayed in the absence and in the presence of isoprenaline; (2) the reduction of activity in the absence of isoprenaline is much more marked than in its presence; and due to this, (3) the relative stimulation of adenylate cyclase activity by isoprenaline ('fold stimulation') is increased. In the case shown in Fig. 1, the stimulation of adenylate cyclase activity by isoprenaline is 1.8-fold in the β AR/ N_s /C vesicles and increases

to 7-fold in the β AR/ N_s / N_i /C vesicles. In a series of 11 experiments the 'fold' stimulation of the enzyme by isoprenaline was 1.32 ± 0.07 in the absence and 3.61 ± 0.6 in the presence of N_i . We have documented that the effects of N_i in the reconstituted system were not the result of preferential reduction of the basal (GTP) activities due to a reduced incorporation of adenylate cyclase or N_s , or of any change in the efficiency of β AR reconstitution (data not shown).

Figure 2a demonstrates clearly that the ability of N_i to increase the relative stimulation of adenylate cyclase activity by isoprenaline is a dose-dependent phenomenon. At the highest level of N_i incorporated, the adenylate cyclase activity assayed in the absence of isoprenaline was inhibited by ~85%, while activity measured in the presence of isoprenaline was inhibited by ~42% (Fig. 2b); this results in an increase in the stimulation of adenylate cyclase activity by isoprenaline from ~1.1-fold for control vesicles, to 4.1-fold in vesicles containing N_i . The mean inhibitions of basal and isoprenaline-stimulated activities by N_i were, respectively, $76 \pm 3\%$ and $43 \pm 4\%$ ($n = 11$). In a separate series of experiments using the resolved subunits of N_i or transducin, we have found that the activities of N_i described here are essentially properties of the $\beta\gamma$ - rather than the α -subunit (manuscript in preparation).

Our results indicate a dual role for the inhibitory nucleotide binding regulatory protein (N_i) of the adenylate cyclase system, one being the mediation of inhibition of adenylate cyclase activity, the other being a tightening of the coupling between adenylate cyclase activity and stimulatory hormones by the marked attenuation of the basal (N_s -mediated) adenylate cyclase activity.

Moreover, not only do our results suggest a dual role for N_i , they also imply two distinct functions for the stimulatory hormone/receptor complex. The first is to accelerate the actual activation of N_s . The second is to suppress the inhibitory action of N_i on GTP-activated N_s . Evidence for the first function derives from studies in which it has been shown that β -agonists promote the interaction between the pure β AR and N_s in phospholipid vesicles, leading to increases in the rate of GTP γ S binding to N_s and the turnover number for the GTPase activity^{7–11}. Evidence for the second function of the hormone/receptor complex is provided by the present work, which indicates that addition of agonists to vesicles containing β AR/ N_s / N_i /C markedly relieves the N_i inhibition, leading to a significant increase in hormonal stimulation.

Of further interest is the fact that purification studies have indicated that in native membranes there is 4–10 times more N_i than N_s (ref. 12), this agrees well with the relative ratios of N_i / N_s (4–5 times) required in our reconstitution systems to both inhibit GTP-activated N_s and enhance the relative stimulation by hormone.

The specific mechanisms by which N_s and N_i are activated under physiological conditions (that is, in the presence of stimulatory and inhibitory receptors, GTP and in a lipid milieu) still remain to be delineated. Both N_s and N_i are heterotrimeric in structure ($\alpha\beta\gamma$) and in detergent solution their activation by non-hydrolysable analogues of GTP involves subunit dissociation into α_s (ref. 13) or α_i (ref. 14), and an intact $\beta\gamma$ species J.C., J. D. Hildebrandt & L.B., unpublished results. It has been suggested that it is the α_s /guanine nucleotide complex which stimulates adenylate cyclase activity. However, the mechanism by which inhibition occurs has been a more controversial subject. It has been suggested that it is the generation of $\beta\gamma$ following N_i activation (and dissociation) which effectively complexes activated α_s (refs 1, 14), while other studies in N_s -deficient S49 *cyc*⁻ cells suggest a direct inhibitory action of α_i on adenylate cyclase¹⁵. Recent studies using phospholipid vesicle systems similar to those described here indicate that bovine brain N_i or bovine retinal transducin inhibit the N_s -stimulated adenylate cyclase rather than the intrinsic activity of the enzyme itself¹⁹. Moreover, experiments using the isolated subunits of these proteins indicate that the $\beta\gamma$ subunit complex is primarily

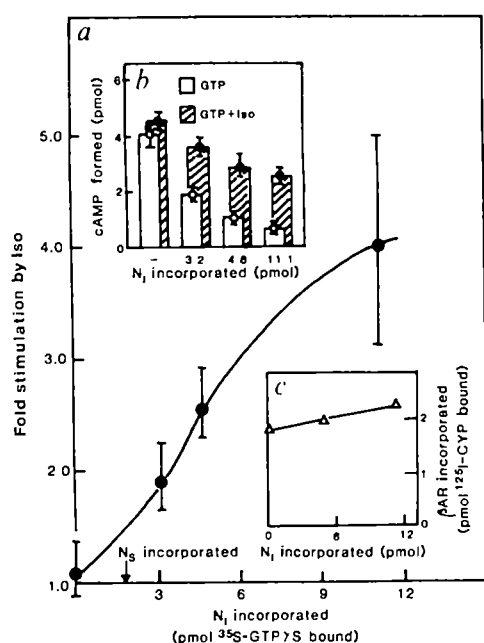


Fig. 2 *a*, Effect of varying N_i on the stimulation (fold) by isoprenaline of the adenylate cyclase activity in phospholipid vesicles. Resolved bovine caudate adenylate cyclase (C) preparations (150 μ l), human erythrocyte N_i (1.16 μ g = 12 pmol), human erythrocyte N_i (0.63 μ g, 1.27 μ g, 3.82 μ g = 6.6 pmol, 13.4 pmol, 40.2 pmol, respectively) and 12 pmol guinea pig lung β AR were added to reconstitution incubations and protein-lipid vesicles were then formed and isolated as described in Fig. 1 legend. The isolated vesicles were resuspended in 0.38 ml of 75 mM Tris-HCl pH 7.8, 1 mM DTT and assayed (20 μ l) for adenylate cyclase activity (30 min at 30 $^{\circ}$ C) as described in Fig. 1 legend. Isoprenaline (Iso) = 20 μ M, GTP = 0.1 mM. The amount of N_i incorporated into the lipid vesicles was determined by assaying 35 S-GTP γ S binding as described previously¹¹. *b*, Effect of varying N_i on the total adenylate cyclase activity in phospholipid vesicles. The experimental conditions were as described for *a*. *c*, Effect of varying N_i on the incorporation of the β -adrenergic receptor into phospholipid vesicles. Experimental conditions as for *a*. The amount of β AR incorporated into the lipid vesicles was determined by 125 I-iodocyanopindolol (125 I-CYP) binding as described previously¹⁸.

involved in mediating the inhibition of GTP- (or isoprenaline plus GTP)-stimulated adenylate cyclase (manuscript in preparation). This would then suggest that a hormone/receptor-mediated release of the system from inhibition by N_i , as documented here, would be due to the complex between hormone/receptor and activated α_s /GTP becoming insensitive to the inhibitory action of $\beta\gamma$.

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Regulation of the association of membrane skeletal protein 4.1 with glycophorin by a polyphosphoinositide

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Many of the physical properties of the erythrocyte membrane appear to depend on the membrane skeleton, which is attached to the membrane through associations with transmembrane proteins¹⁻⁵. A membrane skeletal protein, protein 4.1, is pivotal in the assembly of the membrane skeleton because of its ability to promote associations between spectrin and actin⁵⁻⁹. Protein 4.1 also binds to the membrane through at least two sites: a high-affinity site on the glycophorins^{2,10} and a site of lower affinity associated with band 3 (ref. 11). The glycophorin-protein 4.1 association has been proposed to be involved in maintenance of cell shape^{2,12,13}. Here we show that the association between glycophorin and protein 4.1 is regulated by a polyphosphoinositide cofactor. This observation suggests a mechanism which may explain the recently reported dependence of red cell shape on the level of polyphosphoinositides in the membrane¹⁴⁻¹⁶.

Purified glycophorin, unlike many transmembrane proteins, is soluble in aqueous buffers in the absence of detergents, forming a micellar structure consisting of 14-18 glycophorin monomers with a sedimentation coefficient in the range 7-8S¹⁷. The micellar form of glycophorin varies in its residual intrinsic phospholipid content depending on isolation conditions^{18,22}, but retains the ability to bind protein 4.1. The interaction between micellar glycophorin and protein 4.1 is highly specific and can be used as a method for affinity purification of protein 4.1 (our unpublished results).

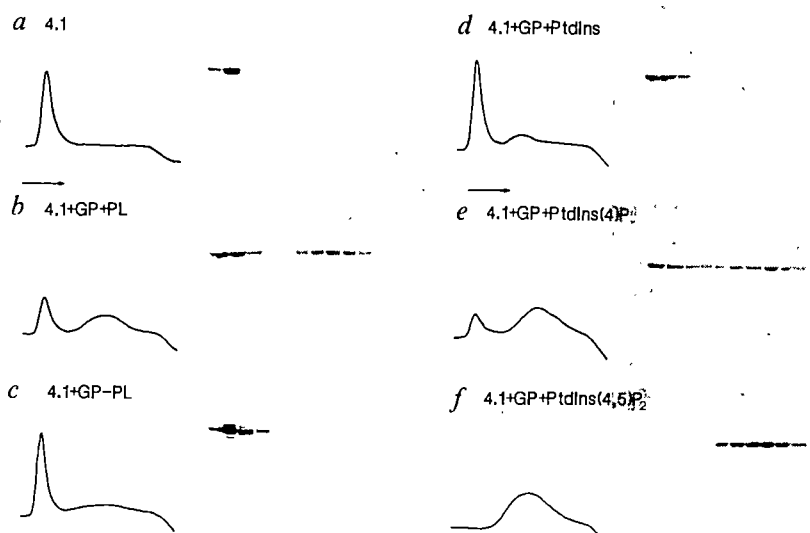
Because of the large size of the glycophorin micelles, the complexes of protein 4.1 and micellar glycophorin could be separated easily from free protein 4.1 by density gradient centrifugation. Micellar glycophorin which retained intrinsic bound phospholipid specifically associated with protein 4.1 (Fig. 1*b*), but this ability was lost on extraction of the bound intrinsic phospholipid (Fig. 1*c*). To demonstrate that extraction of bound phospholipid did not irreversibly denature glycophorin, the extracted phospholipids were recombined with glycophorin; glycophorin reconstituted in this way regained its protein 4.1 binding activity, whereas glycophorin treated identically but without added intrinsic phospholipid, did not bind protein 4.1 (not shown).

To determine whether the association between glycophorin and protein 4.1 required a specific phospholipid, glycophorin free of phospholipid was reconstituted with various phospholipids and its protein 4.1 binding capacity studied. Although all the phospholipids were readily reconstituted into glycophorin micelles, only the polyphosphoinositides restored the ability of glycophorin to bind protein 4.1 (Table 1 and Fig. 1*d-f*). Glycophorin forms a stable mixed micelle with phospholipids, probably by hydrophobic interactions between the acyl chains of the phospholipid and the transmembrane segment of glycophorin. The stability of this complex is demonstrated in Fig. 2*a*, in which glycophorin micelles containing 32 P-labelled polyphosphoinositides were analysed by density gradient sedimentation. The polyphosphoinositides completely co-migrate with glycophorin, indicating that the complex is quite stable. Furthermore, on addition of protein 4.1 the sedimentation coefficient of the glycophorin-polyphosphoinositide micelle increases demonstrating formation of a ternary complex. The polyphosphoinositides show a differential reconstitution of glycophorin's protein 4.1 binding activity (Fig. 1*e, f*). Glycophorin micelles containing phosphatidylinositol 4-phosphate

Fig. 1 Sucrose density gradient analysis of the association of protein 4.1 with glycophorin-phospholipid micelles showing absorbance at 280 nm and corresponding fractions on SDS-polyacrylamide gel electrophoresis (SDS-PAGE). *a*, Protein 4.1 alone; *b*, protein 4.1 plus glycophorin containing intrinsic phospholipid; *c*, protein 4.1 plus glycophorin stripped of phospholipid; *d*, protein 4.1 plus glycophorin reconstituted with phosphatidylinositol (PtdIns); *e*, protein 4.1 plus glycophorin reconstituted with PtdIns(4)P; *f*, protein 4.1 plus glycophorin reconstituted with PtdIns(4,5)P₂. The glycophorin in *c* was treated as in *d-f*, but without added phospholipid.

Methods. Glycophorin was prepared by the lithium diiodosacrylate/phenol method¹⁸, stripped of phospholipid by extraction with chloroform/methanol/12M HCl (200:199:1)²², and desialated². Desialoglycophorin was reconstituted with phospholipids by addition of aliquots of glycophorin (5 mg ml⁻¹) in 60 mM octyl- β -D-glucoside to a dry phospholipid film. The detergent was removed by dialysis, leaving a solution of glycophorin-phospholipid mixed micelles^{2,27}.

The phospholipid content of glycophorin micelles was quantitated by phosphate analysis^{49,50}. Protein was measured by the method of Lowry using bovine serum albumin as a standard^{2,51}. Purified protein 4.1 (refs 5, 24) and or glycophorin at a 1:2 molar stoichiometry was applied to a 5–20% sucrose density gradient. The gradients were centrifuged at 40,000 r.p.m. for 16 h in a Beckman SW-40 rotor. The buffer comprised (in mM); 130 KCl, 20 NaCl, 10 Tris, 1 MgCl₂, 0.5 β -mercaptoethanol, 0.1 diisopropylfluorophosphate, pH 7.6 (isotonic KCl buffer).



(PtdIns(4)P) bound protein 4.1 with lower apparent affinity than micelles containing phosphatidylinositol 4,5-bisphosphate (PtdIns(4,5)P₂). The association between protein 4.1 and glycophorin-PtdIns(4,5)P₂ was abolished by treatment of protein 4.1, with *N*-ethylmaleimide, demonstrating that the binding is specific (not shown).

To determine whether the glycophorin-polyphosphoinositide complex could be a binding site for protein 4.1 on the red cell membrane, we measured the ability of different glycophorin-phospholipid combinations to compete with membranes for

contrast to the results obtained with glycophorin micelles reconstituted with other phospholipids (Table 1). Glycophorin-PtdIns(4,5)P₂ micelles displaced ³²P-protein 4.1 from IOVs more effectively than micelles containing PtdIns(4)P. A plot of the data using the method of Dixon²⁶ indicated that the inhibition of protein 4.1 binding to IOVs is competitive (not shown). Glycophorin-polyphosphoinositide micelles did not block protein 4.1 binding by direct interactions with the membrane, as IOVs did not bind glycophorin-polyphosphoinositide micelles and there was no measurable exchange of ³²P-polyphosphoinositide between micellar glycophorin and the stripped IOVs.

The glycophorin-polyphosphoinositide micelle contains a specific, high-affinity binding site for protein 4.1 which competes with membranes for protein 4.1 binding. To show that glycophorin in a membrane environment also requires the polyphosphoinositides as cofactors for protein 4.1 binding, glycophorin was reconstituted into phosphatidylcholine liposomes together with other phospholipids^{2,27}. We also prepared phosphatidylcholine liposomes containing polyphosphoinositides but no glycophorin, to determine whether protein 4.1 associates directly with the polyphosphoinositides. ³²P-labelled protein 4.1 bound saturably and with high affinity ($K_a > 10^7$ M⁻¹) to phosphatidylcholine liposomes containing glycophorin and PtdIns(4,5)P₂. In contrast, liposomes containing glycophorin and other phospholipids showed only nonspecific protein 4.1 binding. Phosphatidylcholine liposomes containing only PtdIns(4,5)P₂ bound protein 4.1 with low affinity ($K_a < 10^5$ M⁻¹).

The association of protein 4.1 with liposomes containing glycophorin and PtdIns(4,5)P₂ indicates that the phospholipid requirement of glycophorin in the membrane is highly specific for PtdIns(4,5)P₂, which suggests that this complex is a protein 4.1 binding site on membranes. However, if this is the case liposomes containing glycophorin-PtdIns(4,5)P₂ should compete with IOVs for protein 4.1 binding. To study such competition, liposomes were combined with IOVs and competition for ³²P-protein 4.1 binding was measured after separation of IOVs and liposomes by sedimentation through a sucrose cushion (see Fig. 3 legend). In these conditions the liposomes remain in the supernatant whereas the IOVs sediment and are found in the pellet. Analysis of the supernatant and the pellet showed that liposomes containing glycophorin were uniformly separated from IOVs (Fig. 3a) and that protein 4.1 binding to IOVs was

Table 1 Effect of phospholipid reconstitution of micellar glycophorin on inhibition of protein 4.1 binding to stripped IOVs

Phospholipid reconstituted	Concentration of glycophorin (μ g ml ⁻¹) at 50% inhibition of 4.1 binding	Moles of phospholipid/glycophorin*
PtdIns(4,5)P ₂	51	1.11
PtdIns(4)P	115	1.05
Phosphatidylinositol	>400	0.98
Phosphatidic acid	230	0.95
Phosphatidylserine	360	1.05
Phosphatidylethanolamine	>400	0.90
Phosphatidylcholine	>400	0.95
No lipid	>400	0.02

* Mean of three determinations of phospholipid, by phosphate assay^{49,50}, and protein, by Lowry's assay^{2,51}.

protein 4.1 binding. Inside-out erythrocyte membrane vesicles (IOVs) depleted of protein 4.1 were prepared^{23–25} and reconstituted with trace amounts of ³²P-labelled protein 4.1. The ³²P-protein 4.1 appeared to retain complete membrane-binding activity, as over 90% of the labelled protein bound to IOVs or glycophorin micelles and competed with unlabelled protein 4.1 for binding.

Micellar glycophorin containing PtdIns(4,5)P₂ or PtdIns(4)P inhibited protein 4.1 binding to stripped IOVs (Fig. 2b), in

Fig. 2 *a*, Sedimentation profiles of ^{32}P -protein 4.1 (○), glycoprotein reconstituted with ^{32}P -PtdIns(4,5) P_2 (△), and complexes of protein 4.1 and glycoprotein that had been reconstituted with ^{32}P -PtdIns(4,5) P_2 (▲) and ^{32}P -PtdIns(4)P (●). *b*, Inhibition of protein 4.1 binding to stripped IOVs by different glycoprotein-phospholipid mixtures: ●, glycoprotein stripped of phospholipid; △, glycoprotein-PtdIns; ○, glycoprotein-PtdIns(4)P; ▲, glycoprotein-PtdIns(4,5) P_2 ; ■, glycoprotein with intrinsic phospholipid. **Methods.** *a*, Protein 4.1 and polyphosphoinositides were labelled endogenously in IOVs with [γ - ^{32}P]ATP 52 . The polyphosphoinositides were extracted from membranes with chloroform/methanol/12 M HCl(100:200:1), and the ^{32}P -labelled PtdIns(4)P and PtdIns(4,5) P_2 were purified on a neomycin B-glycophase G CPG/200 column 53 . The purity of the polyphosphoinositides was analysed by TLC 53 . *b*, Protein 4.1 binding to stripped IOVs and inhibition of binding were determined in isotonic KCl buffer. ^{32}P -labelled protein 4.1 (3.5 μg) was combined with stripped IOV (60 μg of protein) then glycoprotein was added and the suspension was incubated for 1 h at 4 °C, then sedimented at 30,000g (30 min) through a 10% sucrose cushion to separate free 4.1 from bound 4.1. The concentration of glycoprotein in the IOVs was estimated from the sialic acid content of the IOVs 2 .

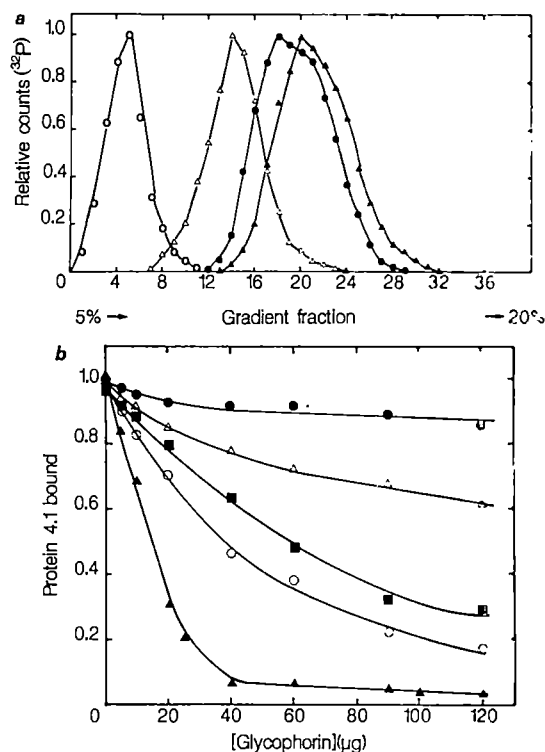
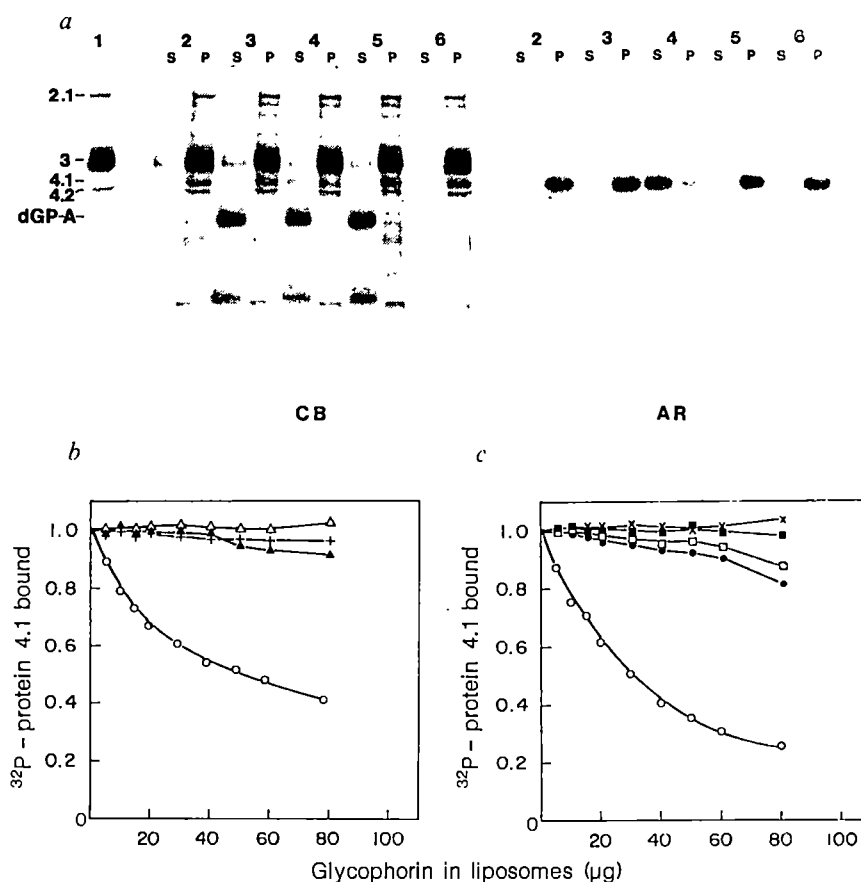


Fig. 3 Inhibition of ^{32}P -protein 4.1 binding to stripped IOVs by phosphatidylcholine liposomes containing glycoprotein, additional phospholipids, or glycoprotein and additional phospholipids. *a*, 7–15% SDS-PAGE (CB, Coomassie blue-stained; AR, corresponding autoradiogram) of supernatants and pellets from a qualitative experiment, showing inhibition of ^{32}P -protein 4.1 binding to stripped IOVs. Lane 1, stripped IOVs; lanes 2, supernatant (S) and pellet (P) of ^{32}P -protein 4.1 bound to stripped IOVs; lanes 3, as lanes 2 except that liposomes containing desialoglycoprotein (dGP) were added to IOVs (the glycoprotein was pre-stripped of all phospholipid before reconstitution into liposomes); lanes 4, as lanes 2 except that liposomes containing dGP and PtdIns(4,5) P_2 were added to IOVs; lanes 5, as lanes 2 except that liposomes containing dGP and PtdIns(4)P were added to IOVs; lanes 6, as lanes 2 except that liposomes containing PtdIns(4,5) P_2 alone were added to IOVs. *b*, Quantitative inhibition of ^{32}P -protein 4.1 binding to stripped IOVs by phosphatidylcholine liposomes containing: dGP alone (+), dGP and PtdIns(4)P (▲), dGP and PtdIns(4,5) P_2 (○), and PtdIns(4,5) P_2 alone (△). *c*, Same as *b*, but phosphatidylcholine liposomes contain: PtdIns(4,5) P_2 and PtdIns(4)P (×); dGP and phosphatidylinositol (■); sGP and phosphatidylserine (□); dGP and phosphatidic acid (●); dGP, PtdIns(4,5) P_2 and PtdIns(4)P (○). **Methods.** Phosphatidylcholine liposomes were prepared by the detergent dialysis method using octyl- β -D-glucoside 2,27 . Phosphatidylcholine (30 mM), desialoglycoprotein (0.12 mM), other different phospholipids at various concentrations, and octyl- β -D-glucoside (340 mM) were combined in an argon atmosphere, then extensively dialysed (5–6 days) against isotonic KCl buffer flushed with argon 2 . After dialysis the liposomes were layered on a 5–20% sucrose density gradient and centrifuged for 22 h at 200,000g in a Beckman SW-40 rotor. Liposomes containing glycoprotein float at 6–7% sucrose whereas free glycoprotein sediments to the bottom of the gradient. The liposome fraction was collected, dialysed against isotonic KCl, and sedimented at 150,000g for 20 min to concentrate and select for larger liposomes. The molar ratio of phosphatidylcholine (PC) to glycoprotein in the liposome after dialysis was 690 ± 90 mole PC per mole dGP as determined by phosphate 50 and protein 51 analysis. The mol% of added phospholipid to phosphatidylcholine before dialysis was: 0.9 for PtdIns(4,5) P_2 , 1.8 for PtdIns(4)P, 3 for phosphatidic acid, 19 for phosphatidylserine, and 10 for other phospholipids. Stripped IOVs (35 μg of protein) were recombined with 1.2 μg of ^{32}P -protein 4.1 (30,000 c.p.m. μg^{-1}). When quantitating the competitive binding of ^{32}P -protein 4.1 between IOVs and liposomes, IOVs with bound ^{32}P -protein 4.1 were combined with liposomes, incubated for 90 min at 23 °C, layered over a 10% sucrose cushion, and centrifuged at 38,000 g for 30 min. In these conditions IOVs sediment through the sucrose cushion whereas liposomes float at the interface of the cushion. When using liposomes lacking glycoprotein in the binding inhibition assay, the same amount of phospholipid was used as in liposomes containing glycoprotein.



inhibited only by liposomes containing both glycophorin and PtdIns(4,5)P₂ (Fig. 3a-c). Furthermore, quantitation of the competition of ³²P-protein 4.1 binding to IOVs demonstrated that liposomes must contain both glycophorin and PtdIns(4,5)P₂ before they can compete for protein 4.1 binding with IOVs (Fig. 3b, c). Liposomes containing glycophorin as well as other phospholipids did not compete with IOVs for protein 4.1 binding. The fact that liposomes containing up to 19 mol% phosphatidylserine (PS) did not compete with IOVs for protein 4.1 binding demonstrates that the association between protein 4.1 and membranes containing PS²⁸ is of lower affinity than that of the protein 4.1-glycophorin association. Interestingly, liposomes containing glycophorin and both PtdIns(4,5)P₂ and PtdIns(4)P are more effective competitors of protein 4.1 binding to IOVs than liposomes containing glycophorin and PtdIns(4,5)P₂ (Fig. 3c). This result suggests that while PtdIns(4)P by itself does not promote glycophorin-protein 4.1 interactions in the membrane, it may have a synergistic effect on the association of protein 4.1 with glycophorin-PtdIns(4,5)P₂.

Thus, the association between protein 4.1 and the glycophorins appears to be regulated by the polyphosphoinositides. While neither glycophorin nor the polyphosphoinositides bind protein 4.1 in the absence of the other, the association of PtdIns(4,5)P₂ with the cytoplasmic domain of glycophorin forms a high-affinity membrane binding site for protein 4.1. The concentration of the polyphosphoinositides in the membrane is maintained by kinases and phosphatases which are specific for each of the *myo*-inositol phosphate monoesters^{7,29-31}. The red cell also has a membrane-bound Ca²⁺-activated phospholipase C which is highly specific for the polyphosphoinositides³²⁻³⁴. The concentrations of PtdIns(4,5)P₂ in the red cell membrane decreases when the cellular ATP concentration falls, or when the Ca²⁺ concentration increases; in both conditions the cell membrane undergoes striking physical changes^{12,15,32,33}. Many hypotheses have been proposed to account for this correlation^{12,15,34,35}; the data presented here provide yet another possible mechanism.

A reduction in the concentration of PtdIns(4,5)P₂ available for binding to the cytoplasmic domain of glycophorin could lead to the release of protein 4.1 from its glycophorin binding site, and hence to its relocation within the cell, possibly binding to band 3 (ref. 11). Indeed, a decrease in the concentration of the polyphosphoinositides as a consequence of metabolic depletion or increased cytoplasmic Ca²⁺ concentration resulted in a dramatic decrease in the ability of stripped IOVs to bind protein 4.1 (our unpublished results).

An exciting implication of this finding is an expansion of the role that the polyphosphoinositides may have in cellular regulation. In most cells the polyphosphoinositides are cleaved by phospholipase C in response to stimulation by extracellular ligands that bind to transmembrane receptors. Cleavage of the polyphosphoinositides results in the release of inositol 1,4,5-trisphosphate and 1,2-diacylglycerol, both of which may act as second messengers^{29-31,36-38}. However, an alteration of the membrane levels of the polyphosphoinositides may also affect the interaction of membrane proteins with other proteins or enzymes, possibly modifying their intracellular biological activities. In this manner, the polyphosphoinositides may play a more versatile part as membrane-bound phosphorylated cofactors. So far, the only example of this potential regulatory role is the interaction of protein 4.1 with glycophorin. However, analogues of protein 4.1 are found in most cells³⁹⁻⁴⁶. In the brain, the protein 4.1 analogue appears to be synapsin I⁴⁵, a protein which is associated with synaptic vesicles and may play a part in the release of neural transmitters^{47,48}. If synapsin I retains functional as well as structural similarity to protein 4.1, the polyphosphoinositides may also have a key role in the interaction of synapsin I with the synaptic vesicles.

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Erratum

Flow law for ice in polar sheets

W. S. B. Paterson; Reply by C. S. M. Doake & E. W. Wolff
Nature **318**, 82-83 (1985)

AN error in *Nature*'s editorial office led to the use of an uncorrected proof for W. S. B. Paterson's contribution to this Matters Arising item. In line 5, the word 'constant' should read 'parameter'. The first sentence in paragraph 3 should read '(2) At Camp Century, the strain rate measured for $\tau_{xz} = 10$ kPa, where x and z define a regular coordinate system, is less than the observational error⁴.' In the address, the postcode should read 'Canada VOP 1HO'.

Geneva meeting the best expected

Only the most unrestrained optimists expected written agreement last week between the United States and the Soviet Union on arms control. It is sufficient that there is to be another meeting.

UNREASONABLE optimism, far from being reprehensible, is more often one of the engines of beneficent change. How else could the New World, encompassing the United States, have been discovered? But unreasonable optimism is an encumbrance when its consequences include unreasonable disappointment, of which there has been far too much in the week following the meeting at Geneva between President Ronald Reagan and Mr Mikhail Gorbachev, general secretary of the Communist Party of the Soviet Union. The fact is that, since high summer, there has not been the slightest chance that the two men in person, however friendly they chose to be, would cut through the difficulties that have defeated their diplomats over several months. The chance that Geneva would bring a specific agreement on strategic arms control had shrunk to nothing by mid-September, and for reasons that had become generally understood. The best that could be hoped for is that the two meeting at Geneva would agree to meet again (see *Nature* 14 November, p.91). In reality, they have done twice as well as that, and have agreed to meet again in 1986 and 1987. It would have been a bad business for all of us if this had not come about. So it is reprehensible that so many newspapers and other sources of public comment should be saying that nothing happened at Geneva. That the two sides did not fall out is what happened.

Where things go from here is the absorbing question. The statements at the end of the Geneva meeting are fuller and emptier than might have been expected. The two superpowers have gone out of their way, with the review conference of the Non-Proliferation Treaty successfully just behind them, to reaffirm their opposition to the spread of nuclear weapons, but have not explicitly said that they will honour, let alone strengthen, the Anti-Ballistic Missile Treaty, the properly ratified agreement on arms control threatened by some of the statements in favour of anti-ballistic missile defences put out from the United States in recent months. Similarly, nothing was said about what will happen when the SALT II treaty (unratified) expires at the end of the year; presumably the calculation is that this question is subsumed in the declaration that the permanent negotiators on arms control at Geneva should accelerate their search for an agreement on strategic arms. Meanwhile, the Soviet side makes no secret of its distaste for US plans for the Strategic Defense Initiative, while the United States can claim some credit for having been allowed to stand firm.

What, then, will happen next? The most urgent task to be tackled once the negotiators reassemble at Geneva is that they should trim their sails to what their leaders say they have agreed. The goal seems to be a 50 per cent reduction of strategic arms, but it is not clear whether the count is to be made by launchers or by warheads. The difference is easily bridgeable, but could also easily be magnified into an insuperable obstacle. The two leaders, having made their journey to Geneva and having claimed success, have a continuing responsibility to ensure not merely that their negotiators are suitably flexible but that the people not privy to the negotiations, the rest of us, are reassured from time to time by public rehearsals of what the understanding is supposed to have been. Otherwise, especially in openly warring Washington, there is a risk that the objective will be corrupted or misunderstood.

Meanwhile, there is a strong case for paying more explicit

attention to the softer parts of the Geneva agreement, those in which the participants promised fuller cooperation on a variety of issues, cultural and scientific exchanges prominent among them. Such aspirations are commonplace when statesmen meet and have little else to agree about. The backroom boys can afterwards be asked to say what scientific collaboration means, and can draw up more detailed agreements for signature at later meetings. The process is almost a formality. Grave officials of learned academies settle on new numbers to describe what numbers of man-months of reciprocal visits shall be allowed on either side. Lists of priorities are drawn up specifying what each side would like to learn from the other, but with the reservation that nothing sensitive in the military sense should be included. Then, when everything is signed and sealed, it turns out that one side or the other is unable or unwilling to make the agreement work. In the past decade or so, for example, it has usually been impossible for Western academies to fill their quota of man-months allowed in the Soviet Union. It is a step in the right direction that, on this occasion, the United States and the Soviet Union have specified, vaguely it must be acknowledged, thermonuclear fusion as a field in which collaboration might be worthwhile. But, yet again, they have been putting the cart before the horse.

The reality is that the formal agreements reached between academies of countries otherwise hostile to each other are never satisfactory. What is needed in the dealings between the United States and the Soviet Union on scientific collaboration is not a set of quotas and a list of priorities but a mechanism by which scholars in both places can decide for themselves what kinds of collaborative research they would like to undertake. Thus the Soviet Union should (and probably does) know that there are many people in the West who would give their eye teeth to work at some Soviet archaeological site, or have a chance to tackle some geological investigation in the Soviet Union. It goes without saying that there are different fields of science in the United States in which Soviet scientists are eager to participate. The obvious difficulties — that both sides are sensitive about security — are well understood, and could be more explicitly defined. Why not, within such a framework, agree that there should be a pot of money to facilitate exchanges and then agree that the projects on which it is spent should be determined by those directly involved, not by their governments' civil servants? To follow past patterns is to invite failure and, ultimately, to bring science into disrepute unfairly. □

Unity by common fright

Europe's plan to collaborate in high technology seems to have struck a welcoming chord.

EUROPE seems to have made an unexpected discovery about itself. When the government of France issued its rallying call to the rest of Europe that there should be a collaborative programme of technological research and development, it was natural that other governments in Western Europe should be suspicious of the motives. For one thing, France did not hide its belief that such a programme was a necessary counterweight to the US Strategic Defense Initiative (SDI), which cannot but be a power-

ful means of stimulating high technology in the United States; the snag was that many governments had good reasons for not opting out of SDI. But, equally, there was no way of making sure that the plan called Eureka was not another way of advancing French claims to European leadership in technology, or perhaps even a way of persuading other governments to contribute to French advancement. For why else, the sceptics were asking earlier in the year, has France been urging technical collaboration on the rest of Europe at least since 1968, when M. Pierre Aigrain (then in the French government, now a director of the French Thomson company) tried on his government's behalf to persuade the European Communities to joint action? During M. François Mitterrand's presidency, the exhortations to technical collaboration have been even more frequent and insistent.

The surprise is that other governments in Western Europe seem at last to have taken to listening to what the French are saying. After two decades of scepticism, the belief that France may have been right all along has begun to gain ground. So much, at least, appears to have been the impression they created at the ministerial meeting on Eureka held at Hannover earlier this month. What seems to have happened in this economically disappointing year is that Western Europe has become collectively despondent about its technical future. Most governments are trying hard to succeed in high technology; most of them are presented every other day with evidence of how difficult it is to succeed in the face of competition from Japan and the United States. It is not surprising that one participant at Hannover said that European governments are now "running scared".

So, almost by chance, Mitterrand's Eureka will succeed when his and his predecessors' efforts in the same direction have mostly failed. The French president deserves credit for his persistence, the other governments of Western Europe for their new-found realism. But it is one thing to be agreed on a common cause and quite another to be sure of attaining it. The practical question still to be answered is whether mechanisms can be found that will enable Eureka to deliver the prizes its supporters promise. Two features of Eureka have so far been agreed. The framework of collaboration will be wider than the European Communities as such, so as to include Austria, Sweden and Switzerland in particular. And the programme will be supported not by another huge pot of public money, but by the funds of those most likely to benefit, the companies that decide to engage in transnational projects with each other.

On the face of things, it is not obvious why European companies should not collaborate with each other on worthwhile technical projects without France's banner labelled Eureka. Although the European Communities have regulations to prevent anti-competitive collusion by competing companies, these are neither as explicit as in the United States, nor are the penalties of transgression as fearsome. Part of the explanation for the poverty of international collaboration in Europe in past decades must surely be the conservatism of even progressive European industrial companies, many of whom still consider themselves as bitter rivals for the same block of overseas trade, that with the United States in particular. There is a chance that exhortation by itself may change this state of affairs, in which case Eureka may be very cheap. More probably, there are technical projects yet to be devised where public money will help to oil rusty wheels.

European governments should also recognize that the kind of collaboration foreseen by Eureka cannot be the basis of long-term economic success. It may help, in the short run, if more companies collaborate on the development of improved products, but either the agreements will then lapse and the companies will revert to rivalry, or they will find they have a successful product which they can market successfully only through some commonly owned channel, perhaps even an independent company in its own right. They will also discover, on the way to success of that kind, that the most wholehearted collaboration cannot succeed if the products whose development is intended are not sold competitively. The lesson from Japan on this point is clear; Japanese companies have succeeded in the export of

high-quality technological products only because their domestic market is even more demanding than those abroad. So will European governments follow the logic of the enterprise on which they have engaged in Eureka, and agree that success entails the banishment of restrictive practices on public purchasing; of the regulations and unwritten rules by which governments in Europe try to preserve the national identity of their domiciled companies; and of the barriers that persist to the free movement of academics and their students from one part of Europe to another? Unless governments agree that these impediments to efficiency are long-term barriers to success in high technology, Eureka will fail. □

Honest pennies to earn

Britain's environment research council should count again the costs of success.

THE British Natural Environment Research Council may fear that there is no way in which it can win friends, or placate its critics, but there is an awkward contradiction built into the way it is compelled to operate on a shrinking public budget. The framework of the inconsistency dates to the Rothschild reorganization of British civil science in 1971, but the issue has been sharpened only in the past five years. Rothschild, it will be remembered, argued that part of publicly supported research should be regarded as applied research, which should by rights be paid for by the ultimate beneficiaries (called customers), whose proxies would be government departments. So, when the British government accepted the formula, nearly a half of the council's budget (which proportion has since shrunk), was transferred to departments such as agriculture (interested in environmental consequences of fertilizer usage), the environment (interested in the environment) and energy (interested in natural resources). With the passage of time, departmental interests have been attenuated, and their willingness to pay for research has correspondingly declined. So the research council, to keep its establishments occupied, has been forced into the market, selling itself as a research contractor.

Earlier this week (see p.307), the council was rightly boasting of its success in selling its technique of side-scanning sonar to the US Geological Survey, which has commissioned it to carry out a survey of the continental shelf off the United States. Now the technique has been licensed to a British company which will, no doubt, be able to establish a profitable business elsewhere overseas, from which the council will also (rightly) profit. But there are other fields in which the council's success as a contractor for research has not been as welcome. Thus the British Geological Survey, originally established so as to provide the United Kingdom with a knowledge of the rocks and resources on which it rests, has been forced by penury to set up as a contractor for a wide variety of geological survey work, much of it well within the field of competence of private geological survey companies. This is one of the minor bones of contention that will no doubt be dealt with by the committee of inquiry now under way.

The perplexing question is how it can be right, even admirable, that the council should sell its sonar technology commercially while being criticized for competing with private organizations in geological survey work. The superficial answer is that there is a vast difference between a technique (such as side-scan sonar) where there are no immediate competitors and one (such as geological survey) where there are all too many. The more durable answer is that the role of publicly supported research enterprises working in the field of applications should be to stimulate companies working in the private sector, never to compete with them. It is probably a fault of the British economic system that there was no small company eager to build a future around the side-scanning sonar that is now a feather in the council's cap, in which case the council should have been worrying to stimulate a company of that kind. What is needed for the future is a mechanism for telling when public research must stop acting as an entrepreneur, becoming a midwife for others. □

US-Soviet fusion

US still vague about Geneva agreement

Washington

FUSION energy researchers in the United States were optimistic last week that the accord reached in Geneva between President Ronald Reagan and Soviet leader Mikhail Gorbachev on magnetic fusion would lead to increased contacts with their Soviet counterparts. Details of what exactly has been agreed were sparse last week, but the two leaders advocated "the widest possible international cooperation" on development of magnetic fusion power. There are still formidable obstacles, however, in the way of building a joint fusion research reactor.

Fusion research does not rank high

Honour for honoured

THE Technical University in Munich (TUM) is trying to woo back Klaus von Klitzing, winner of this year's Nobel Prize for Physics (see *Nature* 24 October, p.667) by offering him the directorship of a new institute in semiconductor physics. Setting-up costs for the institute are estimated at more than DM20 million, of which it is hoped industry will provide one-third, the other two-thirds coming from the Bavarian state government. Von Klitzing is said to have indicated interest, but is undecided about accepting.

From 1980 to 1984, von Klitzing was an associate professor at TUM but in a reorganization of the physics faculty he was not promoted to a full professorship, so he accepted a directorship at the Max Planck Institute of solid-state physics in Stuttgart. At the new institute, he would be a full professor and will have the opportunity to teach, which he is said to want.

The institute will be set up to provide the most advanced research facilities. It will be divided into three sections, each headed by a director — von Klitzing's colleague Frederik Koch, who proposed the original idea for the institute, has been invited to lead one of those sections. The plan also includes six scientists and nine technicians as well as administrative and secretarial staff. All of these are new positions and have to be included in TUM's next budget. Land is already available but finance for a new building and equipment will be discussed early in December when the state government meets with various industrial concerns.

The institute will be named after the Berlin physicist Walter Schottky. TUM intends to do all it can to lure von Klitzing back, not just because of his recent honour, but because it considers semiconductor physics an important field. Jen Altman

among the issues needing discussion between the superpowers, which perhaps explains the vagueness of the public statements, although further details may yet emerge. It will be for the Department of Energy to draw up detailed proposals for an agenda on collaboration, which could be discussed when Reagan and Gorbachev meet next year in the United States.

There are several reasons why magnetic fusion is a good candidate for international cooperation. Most of the literature is unclassified and the next generation of research machines (which will seek to attain plasma ignition, the point at which fusion becomes self-sustaining) will be enormously expensive.

The US magnetic fusion research budget, though faring well by comparison with other energy research projects, has declined substantially in recent years, from \$464 million in 1984 to \$429 million in 1985, with the budget for fiscal year 1986 down to \$382 million. The programme's goals have correspondingly been pushed into the future. Dr Harold Furth, head of the Princeton Tokamak Fusion Test Reactor, says that because of budget constraints, plans to introduce tritium (the fusion fuel) into the Princeton reactor have been put back until at least the end of 1988. No decisions have been made on a new research machine for the United States in 10 years. Cooperation with the Soviets would be one way to make a limited budget go further.

Soviet magnetic fusion research is comparable with that in the United States. Collaboration takes place at present under an intergovernmental bilateral agreement first negotiated in 1973 and extended in 1983. The number and the standing of scientists in reciprocal exchanges are, however, widely believed to have declined in recent years (partly because of protests over Soviet human rights abuses and its invasion of Afghanistan). There were six scientific teams exchanged last year. Dr Frank Press, president of the National Academy of Sciences (NAS) (whose personal efforts to further individual US-Soviet exchanges have been criticized by anti-Soviet hardliners in the Pentagon) said the Reagan-Gorbachev agreement will affect exchanges "productively".

In the short term, there are likely to be many benefits from increased cooperation on basic plasma physics research and on design and engineering of fusion equipment and diagnostics. But if increasing the frequency of scientific visits is a feasible goal, there remain serious difficulties for cooperation on a fusion reactor. Dr John

Richardson of NAS says that a role can be seen for two machines, one to demonstrate plasma ignition and a second to examine engineering concepts for a future commercial reactor. And there are several ancillary projects that might be tackled as collaborative ventures, such as compact toruses, magnetic mirror fusion and hybrid reactors whose neutron flux would be used to manufacture fuel for conventional fission reactors.

But Richardson can see no "natural division of labour" for a truly international four-way agreement to include Europe, the United States, the Soviet Union and Japan; so far, it has not even been possible to negotiate such a deal with Europe.

Tim Beardsley

Geneva summit

Soviet optimism on fusion plan

THE Soviet response to the prospect that the Geneva summit could lead to increased scientific cooperation has been slower than that in the United States, but is no less enthusiastic. Mr Mikhail Gorbachev himself has been reluctant to "deal with the nuances" of possible cooperation projects, as he phrased it at Geneva last week, preferring to stress the funds that could be released to fight world hunger if the arms race were halted. Indeed, when he did commit himself on a specific project — fusion research — what he had in mind was not bilateral but multilateral cooperation. "It was decided", he said, "jointly to approach a number of other states about cooperation in the field of thermonuclear research. This is a very interesting idea which could guarantee for mankind an inexhaustible source of energy."

The view of the Soviet scientific community was reflected last week in Moscow by Dr Petr Timofeev, deputy director of the Institute of Geology of the Academy of Sciences, who said that the institute had been involved in a number of cooperation projects with the United States on the geology of the oceans which had, regrettably, been curtailed in recent years, but would now, he hoped, be renewed. Other potential fields of cooperation in which Soviet scientists are interested include space research, the "conquest of human disease", the environment (a delegation from the US Environmental Protection Agency visited the Soviet Union just before the Geneva summit) and, of course, controlled thermonuclear fusion.

If this cooperation project does materialize, it could prove a particular blessing to Academician Evgenii Velikhov, head of the Soviet nuclear fusion programme. Velikhov is a great advocate of international cooperation on fusion. It was he who put the proposal for the INTOR international tokamak project to the International Atomic Agency.

Vera Rich

US non-proliferation policy

Pact with China almost ratified

Washington

THE US Senate voted last week to approve President Reagan's controversial nuclear cooperation agreement with China, adding only some less-than-onerous certification requirements the President must meet to ensure that the agreement does not contribute to the spread of nuclear weapons. The Senate's approval is expected to be echoed shortly by the House of Representatives.

Opponents of the agreement have denounced it as a step backwards for the cause of nuclear non-proliferation: intelligence reports have been cited to support the claim that Chinese nuclear engineers have helped Pakistan to achieve a suspected nuclear weapons capability, and there have been reports of nuclear dealings with Argentina, South Africa and Iran. The agreement as signed by the President includes no formal non-proliferation safeguards, and critics say that this will encourage other countries to refuse to submit to non-proliferation safeguards.

The administration's answer is that China need not submit to safeguards because it is already a nuclear weapons state. The administration says that China has provided verbal assurances of its commitment to non-proliferation, but these have not been sufficient to quell anxieties in Congress. As recently as 1978, a Chinese leader publicly stated that all nations had the right to "nuclear strength".

More recently, China's position has changed: it has joined the International Atomic Energy Agency (IAEA) and in September announced for the first time that it would voluntarily open some of its civil nuclear facilities to IAEA inspectors. It will also apply IAEA non-proliferation provisions to its own exports.

Nevertheless, both the Office of Technology Assessment and the Nuclear Regulatory Commission have raised doubts about the agreement since it was introduced to Congress in July. Does it, for example, provide sufficient guarantees in the event of a serious deterioration of relations between the two countries? Although it specifies that prior mutual agreement would be needed before any US-derived nuclear fuel could be reprocessed or further enriched, many question how effective this would be if China wanted to process for weapons use stocks of nuclear fuel already on hand. And the agreement includes no clear US veto rights over exports of weapons-grade plutonium made from US fuel.

The sudden climb-down last week by opponents of the agreement seems to represent a simple recognition of political realities: to demand a substantially changed agreement that would require new negotiations with China would have needed an unlikely two-thirds majority in

Congress. Furthermore, any action would have had to be taken before 11 December, when the agreement is due to come into effect.

The modest requirements attached to Congress's approval are that the President must certify that nuclear technology and materials supplied to China will be used solely for their intended peaceful purpose, and that China will provide unspecified

"additional information" on its non-proliferation policy. In addition, the President must certify that US domestic law will be observed, and that requests for reprocessing approval can be granted or denied individually. The administration has cooperated with legislators on the wording of the certification requirements and is content with them; the way appears now to be open for US nuclear construction companies to compete with their European counterparts for contracts in the Chinese nuclear power programme.

Tim Beardsley

Television technology

Picture compression in sight

Princeton, New Jersey

SQUEEZING more television channels into a given bandwidth, every communication company's objective, is also high on the list of RCA's objectives. The company's research laboratories here are designing a novel electronic test unit that will double the number of television channels that can be carried by a satellite transponder.

The computer-controlled unit will show how well imperfect television signals, with some picture frames entirely missing, can be regenerated at the receiving end. To begin with, the tests will be conducted omitting every second frame of a complete television transmission. They will then be extended to see whether the system will work when more than half the signal is not transmitted.

The technique, called Motion Adaptive Interpolation, relies on a computer algorithm to reconstruct the missing frames. A less refined version of the system has been operating since 1983 on the telecommunications satellite, Satcom V, built by RCA to carry television signals to Alaska. Signals at the receiving end are regenerated from signals from which half the frames are missing, but the quality is poor. RCA considers that substantial improvements would be needed if the system were to be used for television traffic within the mainland United States.

RCA now operates five telecommunications satellites, and plans to launch two more by the end of the year. Roughly half the traffic consists of television transmissions between the major television networks and their affiliated stations and between programme suppliers and cable networks.

Predictably, processing television signals from which every other frame is missing imparts a jerky motion to moving objects. The machine on which RCA is working would store successive frames in such a transmitted signal and then, by interpolation between the positions of moving objects, reconstruct the missing intermediate frame.

If the development proves successful, RCA plan to use the system on its video-conferencing links and would also expect

suitable computing machinery to be installed at the head-ends of cable television networks, at the points where signals are received from linking satellites for onward transmission through the network.

Another spur to these developments is the general concern for the design of television receivers for the 1990s, on which several manufacturers are working. One likely development is that of digital transmission of the information required for each picture element (pixel), which among other advantages, will allow colour and luminance information to be transmitted separately, allowing greater control of picture quality.

Part of the development now under way is psychologically based; RCA wants to know how the human eye perceives motion in a sequence of two-dimensional images. The research group has provisionally worked out a scheme whereby it is not necessary to compare entire television frames to detect motion in one relative to the other. Instead, a sequence of progressively more rudimentary frames is constructed by omitting alternate rows and columns of pixels from a frame; the hope is that it will be possible to identify significant motion in the most rudimentary of these digital pictures, thus reducing the amount of data to be handled by the computers. It may then be feasible to incorporate enough processing capacity into individual television sets.

The same principle of data compression is being used in the development of a "smart" camera which may be used as a means of detecting only significant movement within its field of view.

Used as an automatic surveillance system, the camera is controlled by a modest microprocessor whose limited capacity is nevertheless able to detect movement in the most rudimentary of the sequence of collapsed frames and then to arrange to inspect a more informative frame in the hierarchy on the succeeding cycle of operation. RCA hopes that the US National Aeronautics and Space Administration will use the system as a motion detector in the gravity-free environment of the space cabin.

Bill Johnstone

Palaeoanthropology

All human life is there

EVEN the most hard-bitten hominid-fossil watchers were impressed by the specimens unveiled last week at the Commonwealth Institute in London. Centrepiece of the exhibition "The Human Story" is a sequence of candidates as human forefathers ranging from *Aegyptopithecus* and *Ramapithecus* through the australopithecines to *Homo* in his/her various forms.



An australopithecine, of the same type as Lucy, the skeleton found in Ethiopia by US anthropologist Don Johanson.

The skulls are boldly displayed so that the visitor can touch them, with mirrors beneath allowing examination of the teeth in a way that would make a dentist feel at home. Pride of place among the fossil exhibits goes to the 1.6 million-year-old *Homo erectus* skeleton WT15000, discovered last year by Kamoya Kimeu near Lake Turkana in Kenya and described in *Nature* in August (316, 788-792; 1985). Kimeu was on hand at the opening of the exhibition to explain that the skeleton on display is a replica — very convincing right down to the Kenya Museum accession numbers in black ink — and that the original is locked away in the Kenya National Museum, safe from the hazards of air travel and public exposure. Back in the museum too are more teeth, eventually destined to rejoin the rest of WT15000.

Another famous hominid fossil, "Lucy", is present in skeletal form and also as one of the life-size figures, reconstructed on the basis of the latest anatomical and cultural data. Lucy, or at least, a

female *Australopithecus afarensis* from around 3.2 million years ago, is to be seen reaching up, hypothetically and bipedally, to gather fruit from a bush. It was a hard life for *A. afarensis*, it has been deduced from their fruit-eating ways and tendency to die off at an average age of 22, hence the wasted appearance of the reconstructed figure.

By the time the human line gets to 100,000 years ago, the reconstructed figure looks far meatier in the form of Neanderthal man. Not the clumsy colossus that has stubbornly survived in popular fiction for many years, but a character looking not unlike a modern-day middle-weight boxer, though one who has had a long career of mismatches.

The first question asked by many visitors to the exhibition seems to be "But

when did they become us?", and the exhibits and catalogue give a good guide to the various possible answers. But the question more likely to be in the visitor's mind at the end of the exhibition is "Has it finished?". After the impressive series of fossils and figures, and stone tools you can play with, comes a dash through recorded history and a bank of flickering television screens, with all four UK channels in quadruplicate or more, apparently representing the present. And then some conspicuously blank walls — the future is unknown, you see . . .

The exhibition owes much to sponsorship by IBM and in February it will take to the road, with stays in Amsterdam, Stockholm, Bremen, Paris and thence to Africa for display in Ghana, Nigeria, Senegal and Kenya.

Charles Wenz

"The Human Story" is at the Commonwealth Institute until 23 February 1985. Details from Commonwealth Institute, Kensington High Street, London W8 6NQ, UK

French education

Stoking the university fires

JEAN-PIERRE Chevènement, French minister of education, plans to double the number of potential university entrants in France by the year 2000.

This could make France an extraordinary place in which to be educated in the 21st century, for already there are a million students in French universities, compared with, for example, only 175,000 university students in Britain. In Britain, fewer than 5 per cent of the population are considered likely to benefit from university education; but in France, by the end of the century, 55 per cent of 17-year-olds could have the coveted "baccalaureat" or "bac" that would give them the right to enter university.

Whether they will enter university, however, is another question. Chevènement's plans include extending the bac — or bacs, as there are half-a-dozen different specializations — into more career-oriented and technical directions. Politically, he wishes to extend the opportunity of gaining the cachet of a "bac" to the largest possible number of children (at present only a third attempt it, a fraction he would like to increase to four-fifths by the year 2000); educationally, he wants to see a work-force better oriented to the demands of 21st century technology. Thus by 2000, the plan is that many who succeed at the bac will not be going to university, although they will have the right, but straight into work, in a new, high-technology France.

Hence if enough work exists in 2000 (at present many unemployed French school-leavers spend a year coasting in university), the universities need not be much larger by that date. But Chevènement plans to increase the number of qualified school leavers to cope with the changing teaching

load, and a figure of 4,000 new teachers a year has been mooted. Some 400 new schools are also to be built.

Further, Chevènement is to loosen the stranglehold of mathematics on French education, much to the disgust of the mathematicians who consider that mathematical ability is essentially a test of intelligence. Thus the volume of mathematics teaching even in the coveted bac-C (mathematics and physics) is to decrease, and in other bacs it will become less abstract and oriented more to the needs of the central disciplines. (For example, in the "management" bac, bac-B1, mathematics will emphasize statistics and economic calculations.)

Among French scientists, reaction is mixed to the proposal to "dilute" mathematics. Biologists favour the move, claiming that many potential biologists are swamped in school by the mathematical demands of the science bacs; but physicists and mathematicians fear a fall in student quality. Chevènement, for his part, has no desire for the number of potential French Fields Medallists to fall.

Chevènement's planned school reforms cannot take effect before autumn 1986, however, by which time a right-wing government will probably be in power. Nevertheless, the minister's plans may not be forgotten. Many of his educational reforms, including bringing back the Marseillaise and improving discipline in primary schools, have gone down better on the right than the left. And Chevènement, once the darling of the left, has made no secret of the fact that he would accept an offer to continue his work, even under a right-wing prime minister. An imaginative conservative government might just agree.

Robert Walgate

US defence research

Will Congress cut SDI?

Washington

AN apparently unending series of exposures of fraud and waste in the Department of Defense (DoD)'s procurement division, coupled with anxiety over the federal budget deficit, is prompting Congress to take a tough line with defence spending this year. One of the casualties is likely to be the Strategic Defense Initiative (SDI), President Reagan's programme of research into anti-ballistic missile defences. Non-SDI research, however, is blooming.

The President's request of \$3,700 million for SDI in fiscal year 1986 (which began on 1 October) has been rudely refused by the House of Representatives, which has offered a mere \$2,500 million. The Senate has not finally made up its mind, but the figure of \$2,960 million has been urged by a committee. (The figure in 1985 was \$1,400 million.) Despite the apparently large increase from 1985, the SDI organization is far from happy, and has made plain to Congress that support at the level that now seems likely will lead to delays of a year or more to key demonstrations.

But if strategic defence research is now in question, other basic research supported by DoD is likely to increase substantially in 1986. This budget item request (known as line 6.1 to insiders) was \$971 million, which is likely to be met or even exceeded. Last year the figure was \$860 million. Historically, roughly half of the 6.1 budget, and some of the more applied 6.2 research, has been spent in universities. The increase for basic research continues a trend established more than ten years ago. This year, however, the Department of Defense has a new pot of money for university research that it is asking to have filled, the University Research Initiative (URI). Responding to widespread concern about the state of science education and research at US universities, the department went to Congress to ask for \$25 million for URI in 1986; the programme would support fellowships and the like at DoD laboratories as well as university research in risky but potentially profitable areas such as materials and structures, fluid mechanics, biotechnology, communications and optical networks. Congress was so impressed with the proposal that a Senate committee voted a total of \$100 million for URI (and DoD has quickly decided that it will, after all, be able to use the lot). This is on top of a \$30 million per year university instrumentation programme and \$500 million of direct research support last year. Even Colonel Donald Carter, acting head of DoD's research and advanced technology division, one of whose jobs is to drum up support for DoD university research on Capitol Hill, admits that

"we're doing quite well this year". But the House of Representatives has so far agreed only to the original request for \$25 million; a compromise will be worked out with the Senate next month.

Universities, despite their perpetually uneasy relationship with DoD because of friction over the issue of research secrecy, are not unnaturally delighted that DoD is "taking the university research infrastructure seriously", according to Robert Rozenzweig, president of the Association of American Universities. But the high-principled association still objects to the addition of inoffensive sounding amendments to other legislation, including DoD appropriations, that seek to provide funds for specific research projects or facilities at particular institutions, usually within the constituencies of the sponsors of the legislation.

Pork-barrel legislation has a distinguished history in the United States. Some 34 projects were supported in this way between 1983 and 1985, according to data

compiled by the association. But Rozenzweig and Robert Clodius, president of the National Association of State Universities and Land-grant Colleges, say that if allowed to proceed unchecked, direct funding of research facilities which circumvents peer-review procedures will "undermine the very system that has made our research enterprise the envy of all other nations". The presidents are lobbying congressional committees to eliminate all pork-barrel amendments.

Among the beneficiaries of current proposed pork-barrel amendments (in a number of different bills) are: Syracuse University (\$12 million); Oklahoma State University (\$1 million); Rochester Institute of Technology (\$11.1 million); Northeastern University (\$13 million); University of Nevada (\$3.5 million); University of South Carolina (\$4 million); East Michigan University (\$2 million); University of Missouri (\$450,000); New York University (\$2.6 million); Tufts University (\$1 million); and Pennsylvania State University, the University of Minnesota and Massachusetts Institute of Technology (each to receive one-third of \$1.7 million).

Tim Beardsley

Japanese audiotechnology

Renoir returns from the dead

Tokyo

IF you want to hear about the Impressionist school of painting, who could be better to listen to than Pierre Auguste Renoir who, along with Monet, was its most famous exponent? The only problem is, of course, that Renoir died in 1919. But thanks to a little modern computer technology this proves to be a trifling objection — at least in Japan.

To help advertise a major exhibition of the Impressionists being held in Tokyo until the middle of December, callers can hear about the philosophy of Impressionism from Renoir himself by dialling Tokyo 320-3000. The voice is the product of the Japan Acoustics Research Laboratory and its construction relies both on the ability to analyse and manipulate sound patterns with the aid of computers and the ability to predict fundamental voice characteristics through knowledge of an individual's anatomy.

According to the institute's director, Dr Masumi Suzuki, many of the major characteristics of a voice are governed by the structure and shape of the oral and nasal cavities and the resonances they produce in air set vibrating by the vocal cords. Of course, regional accents and the like are not shaped only by such parameters, but the characteristics that enable one to recognize a voice independently of its accent are. The structure and shape of the oral and nasal passages can themselves be measured relatively easily from an X-ray. Where such data are not available, they can be predicted with less accuracy from de-

tailed measurements of the face and neck.

Once the details of the vocal tract are available, simulation proceeds through use of a computer model. A human voice is input (reading French in Renoir's case) and analysed spectrographically. A computer model of the effect of the different resonances produced in different regions of an individual's vocal tract is then used progressively to modify spectral components of the input voice. The result, in Renoir's case, is a new voice that still, according to native French speakers, is perfectly accented French but has (lacking evidence to the contrary) acquired the characteristics of Renoir's voice.

But the "science" of voice analysis does not stop there. If a voice can be predicted from a face, why not a face from a voice? Some researchers have had a try with one of Japan's most wanted criminals, the self-styled "man with 21 faces" who extorts money from confectionery manufacturers by placing poison in bars of their chocolate on sale in supermarkets. All that is known about him is the sound of his voice on tapes and recorded calls. Despite the production of a portrait based on his voice, however, his arrest has moved no closer.

Patriotic Americans are not forgotten either in famous historical figures whose voices have been brought back from the dead. One can hear Abraham Lincoln proclaiming "Fourscore and seven years ago our fathers brought forth on this continent a new nation . . .", in tones to which his high and slightly bent nose apparently contributed a great deal. **Alun Anderson**

UK computing

Prospects good and bad

By the United Kingdom's own rather sad standards, computing research in universities is doing relatively well. Sir Keith Joseph, Secretary of State for Education and Science, this month gave the Computer Board for Universities and Research Councils an extra £1 million for 1986–87, to be followed by £2 million for each of the next two years, to enhance Janet, the wide area network that will connect computers in all universities, research council sites and polytechnics. Although the demand by researchers for computing facilities far exceeds resources, the Computer Board continues to make a relatively successful case to the government for increased support, most recently in its three-yearly report (see below).

In the private sector, computer companies themselves are finding life difficult, but the manufacturing industries are greatly increasing their use of computers. A survey of more than 2,000 engineering companies, sponsored by the Department of Trade and Industry and published this week*, shows that nearly 60 per cent of

UK board reports

THE report of the UK Computer Board for Universities and Research Councils, published this month (Cmd 9671, HMSO, £3.30), covers the period April 1983 to March 1985. During this time, the board was mainly concerned with supporting local computing facilities for research and teaching in universities, supporting national facilities and promoting the efficient sharing of resources. The most important developments were the extension of supercomputing facilities in the two national centres and the development of Janet, the wide area network connecting all research establishments. The board has also taken a lead in developing standards and encouraging the use of computers for teaching in universities. Reappraisal of some of its methods has been caused by its observations of developments in the United States and Japan.

During the next few years, the board will re-examine its relationship with the University Grants Committee (UGC) and will assess its priorities for what it sees as the two ends of a spectrum — supercomputers for the specialized research user and workstations for the undergraduate user.

A joint working party with UGC and the Advisory Board for the Research Councils has set up two subgroups; one, chaired by Professor A. MacFarlane (Cambridge) will examine the scientific need for advanced computing facilities and the other, chaired by Professor E. Spratt (Kent) will examine technical options for meeting that need, particularly in collaboration with industry.

Maxine Clarke

UK engineering plants are using or are about to use computers. In 1986, the industry plans to spend £250 million on software and £600 million on hardware, mainly on minicomputers and mainframe computers rather than micros. John Butcher, Under Secretary of State for Industry, says that the United Kingdom may be leading Europe in the uptake of computers for engineering purposes, "although European data are hard to come by".

The most widely used applications of computers in industry are in manufacturing management and mechanical design, although computer-aided design and draughting are the fastest-growing applications. The top-selling microcomputers are IBM personal computers, XTs and ATs; Apricot is a poor second with only half as many installations as IBM.

IBM is the largest manufacturer of computers in the United Kingdom, and, according to a forecast published last week*, although the company's activities contribute to import savings, UK computer companies are growing too slowly. "The prospects for ICL [Britain's nationalized computing company] are less clear now that the government has largely abandoned its 'buy British' policy and the outlook for small computer companies is extremely volatile", according to Cambridge Econometrics' model. The deficit on the balance of payments for office machinery and electronic data-processing equipment rose from £200 million to £1,100 million between 1980 and 1983 (current prices).

Cambridge Econometrics expects industry's investment in computing equipment and export demand to increase by 4 per cent per year for the next 10 years; by the end of the century, the demand for office machines and data-processing equipment will still be the highest for all manufacturing industry in Britain. But British companies are unlikely to be taking advantage of these opportunities.

The study suggests that new domestic and foreign entries to UK production during the next few years may improve the viability of the industry, but it says that "we do not see enough evidence at the moment to build such hopes into our central forecast". Large companies at present involved in other areas of electronics, particularly military applications, could enter the civilian computer market, or more foreign companies could locate European production centres in Britain. But these remain hopes rather than predictions.

Maxine Clarke

*Third Annual Engineering Computers' Survey in Engineering Computers, November 1985; Prospects for Computers and Office Machinery in the UK to the year 2000 Cambridge Econometrics forecast 85/2. Price £500.00 from PO Box 45, 21 St Andrews St, Cambridge CB1 2EX, UK.

UK space centre

Space programme coordinated

BRITAIN last week re-entered the international space arena with the announcement of its National Space Centre (NSC), to be based in London. The first director general of the centre is Roy Gibson, who was also the first director of the European Space Agency (ESA), for a three-year period. NSC's initial budget is £100 million a year, or about one-fifth of the French government's space budget.

The purpose of the centre, a concept that the Minister for Industry Geoffrey Pattie has taken the lead in promoting, is to coordinate Britain's fragmented space programme, which at present includes projects sponsored by the Department of Trade and Industry (DTI), Ministry of Defence (MoD), Science and Engineering Research Council (SERC) and Natural Environment Research Council (NERC). But "no change in policy" has occurred with respect to ESA; NSC is intended to make Britain's contribution "more efficient".

The first task for NSC is to develop a long-term strategy for Britain's space effort by coordination of industrial, defence and scientific users of space, who will be able to influence the policy of the centre by "financial and in-kind contributions". NSC's first projects will be based at the Royal Aircraft Establishment, Farnborough (where DTI and MoD already have programmes) and at SERC's Rutherford Appleton Laboratory, the present focus of the academic space programme in Britain.

Professor Bill Mitchell, chairman of SERC, welcomed the formation of NSC last week on behalf of SERC and NERC. The main interests of the research councils are astronomy from outside the atmosphere and remote sensing. SERC, NERC and DTI already collaborate on the latter.

The centre will not be involved in the US Strategic Defensive Initiative "at this stage" — the largest proposals with which it is at present concerned are whether, as a member of ESA, to take part in the US space station planned for the 1990s and whether to support the development of the novel reusable launch vehicle *Hotol* in collaboration with the private sector.

Britain pulled out of Europa, its collaborative space venture with France and Germany, 25 years ago. Since the failure of that project, France has taken the lead in the development of Ariane, now a commercial competitor of the US space shuttle. Although Britain's effort has a long way to go before catching up with other countries with space programmes — not only France and the United States, but also Japan, West Germany, Italy and India — the creation of NSC is a step in the right direction.

Maxine Clarke

AIDS in Japan

No screening of blood donors

Tokyo

OVER the centuries, Japan has fought off foreign invaders with a fervour second to none, but in the face of an invasion of AIDS (acquired immune deficiency syndrome), Japan has been slow to protect the purity of the national blood. The AIDS virus has already made significant inroads into the blood of certain sectors of the Japanese population. Yet there are still no plans to introduce routine screening of blood donors as in the United States and Britain.

The number of confirmed cases of AIDS in Japan has risen to eleven since March, when the first two cases were reported among haemophiliacs (*Nature* 315, 8; 1985). Six of the eleven were or are homosexuals and five haemophiliacs. Two of the homosexuals and four of the haemophiliacs had died by late October according to the Health and Welfare Ministry.

Many more Japanese are known to have been exposed to the virus. In September, a team at Jutendo University headed by Takao Matsumoto found that the blood of 5 out of 103 homosexuals contained antibodies to the AIDS virus, HTLV-III (human T-cell lymphotropic virus), while in October the Health and Welfare Ministry revealed that, of 395 haemophiliacs surveyed across the nation between September 1984 and this summer, 120 or 31 per cent have been exposed to AIDS, probably through treatment with blood plasma products from the United States. With about 5,000 haemophiliacs and 300,000 homosexuals in Japan, estimates of those already exposed to AIDS runs into tens of thousands.

In response, the Health and Welfare Ministry has announced plans to double the amount of blood taken from individual donors to reduce dependence on imported blood plasma (*Nature* 317, 101; 1985), but earlier this month, in reply to an inquiry by a communist member of the House of Representatives, the ministry said there is at present no need for mandatory AIDS screening of blood donors. Instead, "voluntary" testing will be covered by the health insurance system for "high-risk" groups such as homosexuals and drug addicts. But in Japan, where pressure to conform is severe, a rush of volunteers can hardly be expected.

Blood donors in Japan are routinely tested for hepatitis virus and venereal disease, which may have induced homosexuals to give blood in order to get free VD checks. A month after the release of the findings by the team at Jutendo University, the Health and Welfare Ministry directed the Red Cross Society to stop accepting blood from homosexuals.

Amid calls for mandatory AIDS tests for blood donors come appeals for the

introduction of screening for another less notorious but equally insidious virus, HTLV-I, which causes ATL (adult T-cell leukaemia). Yorio Hinuma of Kyoto University's Institute for Virus Research estimates that a million Japanese are now carriers of ATL virus. Although the normal infection route is from husband to wife and from mother to child, Hinuma suspects that each year as many as 40,000 Japanese may be "artificially" infected with ATL virus by blood transfusion. Only one in a thousand carriers usually develops the disease but for those who do, ATL is almost invariably fatal, with 50 per cent succumbing within six months and the rest within two years. Professor Hinuma stresses, however, that there is very little relation between the ATL virus HTLV-I and the AIDS virus HTLV-III, and he considered the HTLV-I, II, III terminology proposed by Dr R.C. Gallo, chief of the tumour cell laboratory of the US National Cancer Institute, to be inappropriate.

As in the case of AIDS, the Health and Welfare Ministry has turned down requests for mandatory screening of blood donors. In both cases, the cost of screening the 8 million people who donate blood in Japan each year appears to be the major deterrent.

David Swinbanks

More AIDS money

Washington

ALARM over the spread of acquired immune deficiency syndrome (AIDS) was evident in an agreement of funding for the US Public Health Service reached last week in Congress. The report established a special fund of \$70 million for combating the disease to be allocated by the director of the National Institutes of Health (NIH); this is in addition to the \$120 million the administration had requested for AIDS research at NIH. The total sum appropriated under the agreement would be \$5,501 million, an increase of 7 per cent over last year's figure, with 6,100 new and competing extramural research grants — substantially more than the 5,000 the administration wanted. The total support for AIDS research throughout the Public Health Service is expected to be more than \$300 million.

The agreement, which has not yet been formally accepted by either arm of Congress, notes that there is an urgent need for more basic research on AIDS and directs NIH to spend "a substantial portion" of the extra \$70 million on basic research. The director of NIH is urged to convene a special working group of both intramural and extramural researchers to decide priorities for such research, which would also report to Congress.

Tim Beardsley

Soviet spaceflight

Medical hazards to cosmonauts

THE illness of Vladimir Vasyutin, which led to the premature return to Earth of the latest long-stay crew of Salyut-7, has been provisionally diagnosed as "an inflammatory disease that did not respond to the drugs available on board". Although Vasyutin was unable to act as commander during the landing, he was well enough to give a brief television interview after touchdown. The doctor who gave him a preliminary check-up said that his condition was "satisfactory", but that he would nevertheless need hospital treatment.

Valerii Kybasov, himself a cosmonaut, told a TASS correspondent that it was decided to bring Vasyutin back to Earth simply because "in our country, man comes first". There have been some hints in the Soviet media, however, of fears that the malady might have spread to his fellow crew members, Viktor Savinykh and Alexandr Volkov. An unnamed official, interviewed at the landing site by Moscow radio, spoke with some concern of an ordinary cold having developed "into some new forms", requiring "urgent intervention from Earth. Both Savinykh and Volkov proved to be in good health, however.

This is the first major medical incident in almost 25 years of Soviet manned spaceflight, although there have been cases of toothache and colic which responded to the on-board medicine chest. Even if Vasyutin's indisposition proves relatively minor, his hospitalization could have important repercussions for the Soviet space programme. The lobby within the Soviet space programme pressing for a flight to Mars claims that the long-term Salyut missions have already established the human organism's adaptation to prolonged weightlessness without permanent after-effect. Vasyutin's illness will provide the "conservatives" who oppose the lobby with a powerful example of their main counter-argument, that space medicine has not yet reached the stage where cosmonauts can be allowed to venture out of reach of a base hospital.

Vera Rich

● Two Syrian trainee cosmonauts have arrived in the Soviet Union to prepare for a future joint mission. This news has caused some speculation among Baikonur-watchers, since even a few months ago, Soviet space officials were emphatic that no further "international" flights were being planned. Even more curiously, the news was announced in *Krasnaya Zvezda*, the Red Army daily. It has been suggested, therefore, that the invitation to Syria to participate in such a flight may be a sop, not unrelated to the tentative political rapprochement of the Soviet Union towards Israel. □

British environment research

From penury to mere poverty?

THE Natural Environment Research Council (NERC), the most friendless of Britain's four science research councils, is hoping that the tide of misfortune is beginning to turn. Mr Hugh Fish, the council's chairman, said earlier this week that he hopes, in the next two weeks, to have assembled the funds (\$2.5 million a year) that will enable Britain to join the new international Ocean Drilling Program being organized by the US National Science Foundation (NSF). The council has also won a modest success in the commercial exploitation of the sea-bed profiling system called GLORIA and hopes that a modest share of the extra £14 million made available for the research councils two weeks ago will allow it to increase its support for university research.

British membership of the Ocean Drilling Program (ODP) has been a thorny issue for most of this year. The hope, now, is that the necessary sum will be raised by means of contributions from industry (mainly the oil companies), government departments and from NERC's own budget. According to Mr Fish, a British delegation has been invited to the next meeting of ODP, in Hawaii in the second week of January, as observers, but he hopes they will be able to attend as paid-up members.

NERC's success with GLORIA (which stands for Geological Long Range Inclined Asdic) rides on a contract with the US Geological Survey last year for the survey of the bottom topography of part of the US economic offshore zone. Now, according to NERC, the contract has been extended to include virtually all of the offshore United States under a six-year contract that will cover, among other things, the development of improved equipment at the Institute of Oceanographic Sciences. At the same time, NERC has arranged to "capitalize on the opportunities presented by this unique British research module" by means of a contract with Marconi Underwater Systems, which have the right to build versions of the asdic system and to provide an underwater survey system overseas. The company apparently expects there to be some £5 million worth of business each year, and is committed to share some of the benefits with NERC.

A statement put out on Monday with the council's annual report for 1984-85 says that the decline of the value of research commissioned by government departments from council institutes, the chief cause of NERC's money problems in recent years, has now been reversed; last year, income of this kind amounted to £24.9 million, compared with £23.7 million in the previous year. Mr Fish said that he hoped the figure will increase as further opportunities for commercialization arise.

But NERC's hopes of winning public support for the costs of restructuring, the research council euphemism for persuading people to retire early, were disappointed; it asked for £2 million, but received only £1.25 million.

Mr Fish and his senior colleagues on Monday repeatedly emphasized how important it is that NERC's research should be commercialized. By means of a sales office in Brussels and an agent in Washington, the council hopes to win extra business from overseas companies and governments, "which is not to suggest that British government departments will not always be important customers". But there are obvious benefits in diversity. "We've had our fingers burned", said one official.

Meanwhile, NERC plans to introduce a more formal system for the assessment of its own activities by the three criteria of timeliness; potential economic and social benefit to the United Kingdom; and the enhancement of Britain's reputation overseas. There is to be a process by means of which the outcome of research decisions will be judged retrospectively by these criteria, with the objectives both of evaluating decisions made and of refining the criteria.

For the immediate future, NERC hopes to increase its support for university research, by about £5 million a year on its present spending, estimated at close on £17 million, counting the cost of research vessels maintained by NERC. Mr Fish says that university research is especially desirable because of its flexibility; people will do "basic research on a short-term basis".

Dr John Bowman, secretary of the council, also said on Monday that there was a case for increasing NERC's interests in atmospheric chemistry, partly so as to make good the loss of support for acid precipitation research some years ago but also for more general reasons. The council also plans "a more sophisticated approach to forestry" and says it has already written to the new director of the National Space Centre (see p.305) to declare its interest in remote sensing of various kinds.

On the research council's future organization, Mr Fish said that a new version of its corporate plan was about to be shown to members of staff for their comments, and would be finally discussed by the council in January. On this occasion, he said, the plan would be more concerned with science than with organization, which would nevertheless not be substantially changed from that in the document published earlier this year.

The council is pushing ahead with the appointment of three scientific directors to oversee the different parts of the council's programme; one is in post and two are

about to be appointed. But it may be decided that these persons should be sited in laboratories and not in the council's headquarters. The future of the British Geological Survey, the largest of the council's dependants, would be decided only after the committee on the subject under Sir Clifford Butler had reported, although Mr Fish gave it as his opinion that the survey needs more money to support its "core" programme.

Last year, the council's total income was £93.4 million, of which £65.3 million was contributed by the general science budget, which supports all the research councils. Although contract research is seen as a growing component of the budget, Mr Fish says that NERC has now learned a lesson from the painful withdrawal of government contracts in recent years, and intends not to employ full-time members of its staff on short-term contract work. □

French experiment on coma patient

FRENCH doctors experimented on a patient who had been in a coma for three years, it was reported in France last week amid a storm of protest. The patient, a young man who was claimed to be "brain dead", has since died, but not, it is claimed, as a result of the experiment, which involved a high-speed blood transfusion into a pelvic bone.

The doctors are reported to have undertaken and announced the experiment to provoke debate on the ethics of such operations, and this objective they have certainly achieved. Thus Louis René, president of the ethical commission of the French medical association, has said he was "profoundly shocked" to hear of the experiment. "Since 1947 at Nuremberg — a symbolic date and place — doctors have affirmed that experiments can only take place with the agreement of the subject", René wrote to the newspaper *Le Monde*. In the case of a patient in coma, this agreement is patently impossible and so experiments on comatose patients should be forbidden, he wrote. Such experiments "treated the patient as an object and denied him all human character", exemplifying "condemnable medical imperialism".

Following this line, Edmund Hervé, minister of health, has demanded an inquiry and cited article 18 of the French medical code which forbids a doctor to subject a patient to unjustifiable risk.

Le Monde, however, quotes a somewhat different view from M. Guy Tiercelin, president of a national anaesthetics commission. "I am not necessarily opposed to an experiment if there is proof of brain death", said Tiercelin. "One could test certain techniques of reanimation, but one must be very vigilant, for we should never perform experiments on patients who may recover".

Robert Walgate

Japanese psychiatry

SIR—The article by Alun Anderson entitled "Abuse for visiting scientists" (*Nature* 315, 361: 1985) contains some noteworthy misunderstandings, and as one of the parties concerned we would like to clarify matters.

Protest action taken against the seventh congress of the Japanese Society on Biological Psychiatry at Gifu was not directed at the visiting scientists from the United Kingdom and the United States. The protest was made in order to obtain open public discussion on the ethics of an experiment on a human fetus carried out by Professor Masayuki Namba of Gifu University and his followers. Professor Namba, the president of the congress, was responsible for the human experiment in his capacity as director of the Department of Neurology and Psychiatry in Gifu University. But he chose to avoid discussion and to cancel the congress.

The Japanese Society on Biological Psychiatry is managed under a highly closed, almost secret system. For example, a non-member who wished to join only for the days of the congress at Gifu had to obtain a written recommendation from one of the board of directors, even though the names of directors and the address of the office are not usually announced. The society has no ethical code governing experiments involving human subjects. We have examined the summaries of papers that were to be delivered at the Gifu congress and past congresses and find evidence of many unethical experiments. So we would like to give a warning about the true nature of the society. The issue of the human experiment at Gifu is now under investigation by an *ad hoc* committee of the Japanese Society of Psychiatry and Neurology but we understand that the doctors involved in the experiment are unwilling to cooperate.

We, the Psychiatrists' Union of Tokyo University, aim to reform the present state of psychiatry in Japan and to that end will take action against unethical psychiatric conduct. A liaison group, including some of our members, has been pressing for open discussion about the Gifu experiment. This group is not, as Anderson wrote, the "antipsychiatrists within Tokyo University". Nor did this group intend to visit the smaller meeting at Nagoya. It is also not correct to describe us as "antipsychiatrists" as there are differences between our views and those of the antipsychiatrists found in the United Kingdom and Europe in the 1960s and 1970s.

Anderson states that there is no evidence of unethical conduct by the outpatients ward doctors of the University of Tokyo hospital. Why is he so dogmatic? Anderson has mentioned the horrific incidents that occurred at Utsunomiya hospital. But the outpatient doctors have long been closely connected with the Utsu-

nomiya hospital as has been widely reported in the Japanese press.

For example, the director of the Utsunomiya hospital set up an outpatient clinic near Tokyo University hospital and the outpatient doctors made use of this clinic for practice and research. Some patients were sent from this clinic to the Utsunomiya hospital. It is a fact that many of the outpatient doctors visited the Utsunomiya hospital to work or for research and contributed to the hospital's development. Many further connections exist.

The miserable state of Japan's mental hospitals as mentioned in Anderson's article is a social problem for the nation. It stems partly from the relationship between university mental hospitals as suppliers of new doctors and other mental hospitals. Many university hospitals treat the mentally ill as research objects and think little of their rights. This trend is closely related to the abuses seen at Utsunomiya where patients were forced to remain in hospital for long periods and were dominated by violent methods.

Furthermore, the government supports this situation through the Japanese Mental Health Act because few legal rights are given to patients, especially those admitted to mental hospitals. Visits from Disabled Peoples International, the International Commission of Jurists, and the International Commission of Health Professionals are necessary and important. These visits are expected to have a favourable influence upon mental health policy in Japan. We regret to say, however, that a large number of Japanese psychiatrists take attitudes similar to the outpatient doctors of Tokyo University. Reform is still far off. To be reconciled with the outpatient doctors is not easy for us at the present.

KIMIO MORIYAMA
(Acting Chairman)

Psychiatrists' Union of Tokyo University,
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Tokyo, Japan

Slighted Dallas

SIR—In his appreciation of the 1985 Nobel Prize award in Physiology or Medicine to Michael S. Brown and Joseph L. Goldstein (*Nature* 17 October, p.569), Peter Newmark has indulged either in the subtlest of *Nature's* ironies or an insult that cannot have been intended by so sympathetic a commentator. Newmark points out that "So far all attempts to lure both of them from their opulent Dallas surroundings to more distinguished institutions have failed". As a longtime admirer not only of our new laureates but of the remarkable medical school in which they work, permit me to suggest that it might be hard, indeed, to identify "more distinguished institutions". Considering the high level of innovative science that is con-

ducted at the University of Texas Health Services Centre in Dallas, I look forward to Newmark's revised estimate of Dallas as more of her sons share the dais with the King of Sweden.

GERALD WEISSMANN

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550 First Avenue,
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Irrigated tomatoes

SIR—I would advise Michael Andrews (*Nature* 17 October, p.570) not to drink water containing 3,800 micrograms of selenium per litre. As Paracelsus said, "the dose alone determines the poison", and a woman who took about 27,300 micrograms of selenium daily for 2 months had toxic symptoms¹. No increase in symptom and disease reporting was found in a population drinking water containing 494 $\mu\text{g l}^{-1}$ of selenium². Andrews does not report the selenium content of the tomatoes he filmed in July, which he wanted to eat. If they were irrigated with water from the California Aqueduct, the water was reported³ in the same month to contain 0 to 2 $\mu\text{g l}^{-1}$. Information on selenium content of common foods is supplied by Underwood⁴.

THOMAS H. JUKES

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Berkeley, California 94720, USA

1. US Public Health Service, Morbidity and Mortality Reports, 33, 157-158 (1984).
2. Valentine, J.L., Kang, H.K. & Reisbord, L. Abstracts, 3rd Int. Symp. on Selenium in Biology and Medicine, Beijing, 26 May 1984, p. 139.
3. Kennedy, D.N. Report of Activities of the Department of Water Resources, Sacramento, California 20 July 1985.
4. Underwood, E.J. (ed.) *Trace Elements in Nutrition* (Academic, New York, 1977).

Evolving journal

SIR—Your review of *Molecular Biology and Evolution* (*Nature* 26 September, p.296) contains two errors. In one, your reviewer asserts that *Molecular Biology and Evolution* "has not expanded since its first issues". The five issues of 1984, the special inaugural issue of December 1983 not being included, totalled 366 pages or 73 pages per issue. The six issues of 1985 totalled 560 pages or 92 pages per issue. That is a 25 per cent increase. That, together with the fact that, in its first year, *Molecular Biology and Evolution* circulation surpassed that of the excellent 14-year-old *Journal of Molecular Evolution*, suggests, contrary to your reviewer's report, that *Molecular Biology and Evolution* has already established itself alongside its worthy competitor.

WALTER M. FITCH
(Editor-in-Chief)

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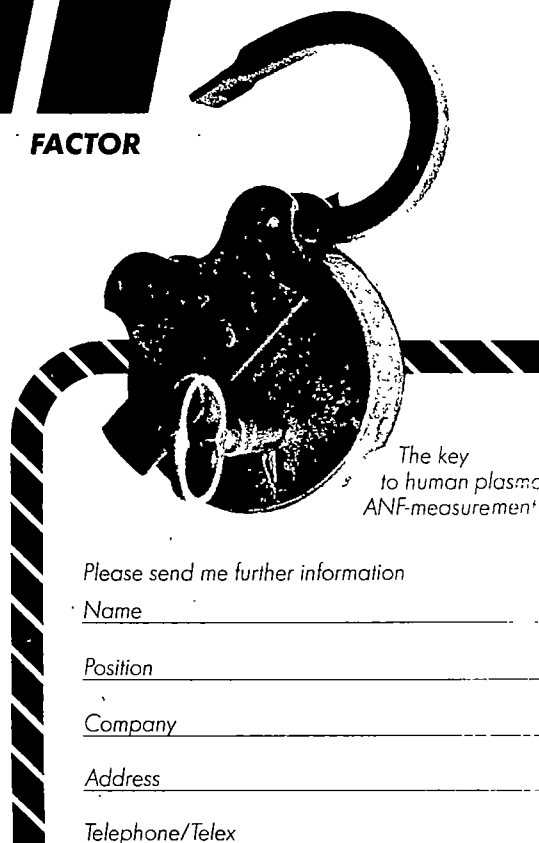
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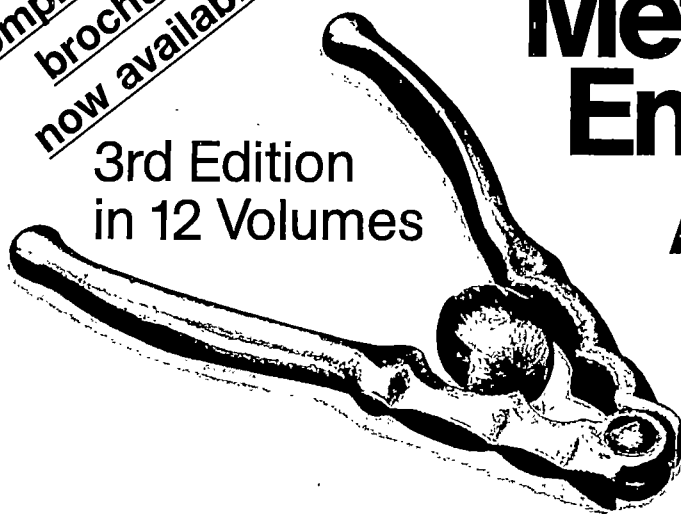
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Testing for cystic fibrosis

It has at last been made on the development of a prenatal test for cystic fibrosis. At first, it will only be useful in some families.

A BATCH of papers published in this issue of *Nature*, together with a paper in the current issue of *Science*, signal the beginning of the end of the hunt for the cystic fibrosis gene, defects of which are responsible for the disease. Although the gene remains to be identified, at least it is now certain that it resides in the middle of the long arm of chromosome 7, within a region that comprises no more than about 1 million base pairs. And the new data offer immediate prospects for the prenatal testing for cystic fibrosis.

Approximately one in twenty Caucasians carry a defective cystic fibrosis gene, and about one child in 2,000 inherits such a gene from both parents and consequently dies of the disease, usually by the age of 30. Despite the frequency of the disease, nothing has been learnt of the gene (or genes) in which the defects arise and no gene product has been convincingly demonstrated. It has not therefore been possible as yet to devise a prenatal screening test for cystic fibrosis relying on the detection of the defective gene or its product. An alternative approach, laid out in detail by Botstein *et al.* (*Am. J. hum. Genet.* **32**, 314; 1980), is to look for and find a restriction-fragment length polymorphism (RFLP) close enough to the cystic fibrosis gene to act as a linkage marker.

An RFLP is no more than a variation in DNA sequence that generates or abolishes a recognition site for a restriction enzyme. Because these enzymes cut through double-stranded DNA, the positions of their recognition sites on a stretch of DNA determine the sizes of the "restriction fragments" of DNA they generate. The chromosomes of any homologous pair are bound to differ by many RFLPs, which can therefore, in principle, be used to tell which of the pair of chromosomes carries, for example, a defective cystic fibrosis gene. If the RFLP is located closely enough on the chromosome to the cystic fibrosis gene not to become separated from it by the normal process of genetic recombination, it will also serve as a linkage marker for the defective gene. Because the RFLP is only a linkage marker, rather than a direct assay of the defective gene, it cannot be used for population screening but only predictively, in family studies.

It has been calculated that if RFLPs are more or less evenly distributed along and among the chromosomes and are

"informative", no more than 150 would be sufficient to provide one closely linked with any gene. So far, however, although several hundred useful RFLPs have been found and many have been localized, they are not evenly distributed and most are informative for only a minority of families. In searching for a linkage gene that could be on any chromosome, it therefore makes sense to begin with the chromosomes that are most saturated with RFLPs and conventional markers (such as different forms of serum enzymes). In this way, by the middle of 1985, several research groups had excluded about 40 per cent of the human genome as the site of the cystic fibrosis gene.

The first sign of success came at the Eighth International Gene Mapping Workshop in Helsinki in August where Hans Eiberg, of the University Institute of Medical Genetics in Copenhagen, reported a tentative link between cystic fibrosis and a form of the serum enzyme paraoxonase (an aryl esterase). Eiberg calculated that the paraoxonase gene and the cystic fibrosis gene lie within ten million base pairs of each other. However, this linkage — now published by Eiberg and others in *Clinical Genetics* **28**, 265; 1985 — was less useful than it might have been because paraoxonase is poorly characterized and the chromosomal localization of its gene is unknown. Rather than dampening the enthusiasm of those who had failed to find an RFLP linkage, Eiberg's result revived their flagging energy.

Among those searching for an RFLP were Lap-Chee Tsui and colleagues at the Hospital for Sick Children in Toronto. In July of this year they came to an agreement to test some of the 200 or so useful RFLPs generated by Collaborative Research Inc. Much to their joy, and purely by chance, a cystic fibrosis-linked RFLP turned up among the first half a dozen RFLPs that Tsui tested. The result was reported at the American Society of Human Genetics meeting in October, and has just been published in *Science* (**230**, 1054; 1985).

What was not reported at the meeting was the chromosomal localization of the RFLP, information that would immediately have enabled others to concentrate exclusively on any RFLPs that they knew to be associated with the chromosome in question. Members of both the Toronto and Collaborative

Research teams say that they had only preliminary evidence at the time and were not prepared to risk presenting a faulty localization in public. Nevertheless, a rumour emerged that their RFLP was on chromosome 7, as they confirm on page 380, although other rumours pointed towards different chromosomes.

Spurred on by the rumours, two other research groups have now found RFLPs that are linked to cystic fibrosis and report them on pages 322 and 384. One of the groups is centred on Bob Williamson's laboratory at St Mary's Hospital Medical School in London. Its RFLP probe is an "anonymous" DNA probe on the long arm of chromosome 7. Williamson's group also reports linkage between the cystic fibrosis gene and two other chromosome 7 genes — that for the T-cell receptor beta chain (p.384) and a collagen gene (*Lancet* 29 November p.1241). The linkage reported by the other group, centred on Ray White's laboratory at the Howard Hughes Medical Institute in Salt Lake City, is to an RFLP that is in the locus of the *met* gene, which is a human oncogene on the long arm of chromosome 7 (see p.285). The overlap between the various probes locates the cystic fibrosis gene in the middle of the long arm of chromosome 7.

Both Williamson's and White's RFLPs are much more tightly linked with the cystic fibrosis gene than is Tsui's. His RFLP is probably no closer than 15 million base pairs (0.5 per cent of the human genome) to the gene. Williamson's and White's are each within about a million base pairs of it, conceivably very much closer. The greater the distance between the RFLP and the gene, the greater the chance that they will be separated by recombination during meiosis, confounding attempts to use the RFLP as a prenatal diagnostic marker of the defective gene. In these terms, Tsui's RFLP is not diagnostically useful, whereas the other two are. What is likely to emerge, when the dust settles, is a set of markers which will make the reliable prenatal diagnosis of cystic fibrosis a reality assuming that all cases are due to a mutation of the same gene.

Both to develop a screening test that avoids the need of family studies and to discover the fundamental basis of cystic fibrosis, vigorous attempts will now be made to identify the gene itself and to find out what it produces. **Peter Newmark**

Evolutionary theory

Hamilton's rule OK

From Alan Grafen

THE darwinian principal of natural selection was extended by Hamilton in 1964 to the evolution of social behaviour¹. This extension is central to much of today's evolutionary biology, but it is something of a sport among mathematical biologists to cast doubt on its validity. Now David Queller, writing on page 366 of this issue², presents a simple and powerful derivation of Hamilton's result that should go some way towards convincing the sceptics.

Hamilton's extension to darwinism is enchantingly simple and has come to be known as 'Hamilton's rule'. In an uncomplicated world, the morphology and behaviour of an individual would affect the number of offspring it had in its lifetime but not the number of offspring other individuals had. Given that individuals do interact, though, the question is how will selection cause animals to value each other's reproduction? Will they place no value on it — that is, will selection favour forms according only to their own number of offspring? Or will they place equal value on their own reproduction and that of others — that is, will selection favour those forms that produce more offspring in total for the species? Hamilton's answer was intermediate between these two ex-

trems. He showed mathematically that natural selection would act so that one individual would value the reproduction of another according to how closely the two individuals were related. Identical twins would value each other's offspring equally with their own; brothers and sisters would value each other's offspring half as much as offspring of their own; and so on. The evolution of a social trait then depends on an 'inclusive fitness' calculation, in which all the effects of the trait on reproduction are added together, weighted by the appropriate relatednesses. Relatedness matters because relatives share more genes than non-relatives, and natural selection is a matter of the nonrandom survival and proliferation of some genes at the expense of others.

Hamilton's rule is simple, but is it true? There is a whole literature (reviewed in more detail elsewhere³) that tries to answer this question. Queller's contribution is to provide a very simple and direct proof of Hamilton's rule, as an antidote to what I feel is the biologically unsympathetic and over-mathematical treatment that is usually applied. The positive feature of Queller's approach is that it sacrifices an element of mathematical rigour for the

sake of deriving a much more general result, and deriving it with clarity and simplicity.

Complete recursion, or dynamic sufficiency, is the element of rigour sacrificed by Queller's approach. In a simple model (one locus, two alleles) in a diploid species, we can say that a rigorous model uses the frequencies of all three genotypes in one generation to predict all three genotype frequencies in the next. Complete recursion is the use of those genotype frequencies for predicting the genotype frequencies in the following generation, and so on. Queller's method has an incomplete recursion, because it uses all three genotype frequencies in one generation to predict the frequencies of the two genes in the next. Without making some assumption

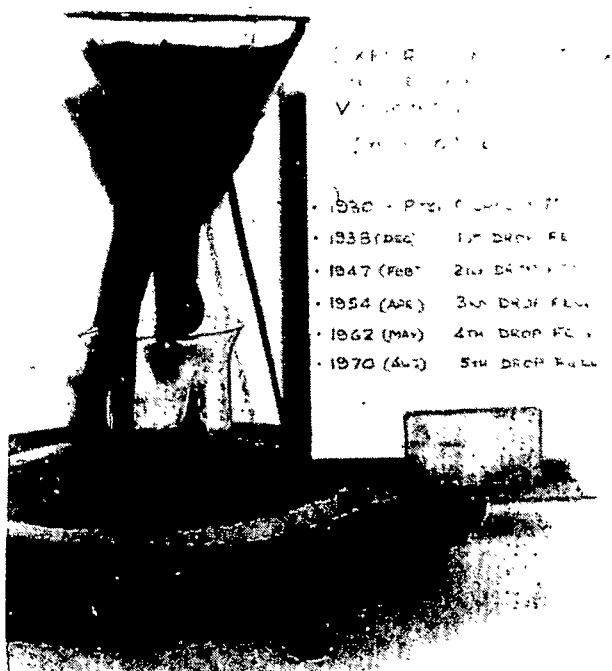
(usually that the Hardy-Weinberg equilibrium holds), genotype frequencies cannot be calculated from gene frequencies, and so it is impossible to apply Queller's equations repeatedly to predict gene frequencies in succeeding generations.

That is the cost of Queller's simplification. Whether it is serious depends on one's point of view. One view is that incomplete recursions are unsafe, because to predict gene frequencies into the future they must make some false assumption (such as Hardy-Weinberg), and since the consequences of the assumption may not be innocuous, the only way to provide a secure argument is not to make the assumption in the first place. But not all conclusions that we draw from evolutionary models depend on predicting gene frequencies in the future. Queller's model predicts whether a character increases or decreases from one generation to the next, and shows that it depends on a form of Hamilton's rule. He makes no claim about dynamic sufficiency (although he does unguardedly claim his model is "exact", a term usually reserved for completely recursive models), nor is any needed for his conclusion to be both interesting and important.

The advantages gained by forgoing complete recursion are great. Since Queller's model does not specify what causes the genetic similarity between donor and recipient, his result holds whether the cause is a recent common ancestry, heterogeneity of the population, or the tendency of individuals to interact preferentially with like genotypes for other reasons. His interactants need not comprise mutually exclusive and exhaustive groups. Completely recursive models are hard work: they assume (contingently, not necessarily) mutually exclusive and exhaustive groups, and they must specify exactly how genetic similarity is caused. For example, a model in which each group comprises half siblings would be quite different from one in which each group was formed by picking individuals at random from two sets of full siblings. Queller's approach shows that the relatedness is one quarter in each case, and so the two are immediately seen to be essentially similar. Completely recursive models would be very different and — though for no very clear reason — would eventually give the same answers as each other (and as Queller's model) in the matters important for the evolution of a character. The models would differ in details of interest to the population geneticist: for instance, they would have different kinds of polymorphic equilibria when the two alleles were so similar in effect as to be empirically indistinguishable.

While, in my view, Queller's trade-off leaves him as a champion of Hamilton's rule in one battle, he has been too ready to concede defeat on its behalf in another. Hamilton's rule was first derived for additive social interactions, in which the

Long-running experiments (I)



Only six drops have fallen, the latest in April 1979, since this experiment was set up in 1930. Its aim is to demonstrate and measure the fluidity and very high viscosity of pitch. The experiment, which continues in the Department of Physics at the University of Queensland, Australia, is one of three long-standing experiments considered in detail in the *European Journal of Physics* 5, 193–200 (1984), along with a request for accounts of further examples.

effects of others on one individual's number of offspring simply added up. What if interactions combine synergistically, as Queller calls it? The problem here is one of measurement. The third, synergistic, term in Queller's form can be made to disappear by agreeing to define benefit and cost as the average effects on individual's fitnesses, rather than as arbitrary terms in a model of fitness¹.

So in Queller's simple model, Hamilton's rule, with costs and benefits correctly understood, is perfectly adequate for deciding the direction of change in gene frequency. In more complex models, in which individuals can pay costs and receive benefit many times in a lifetime, a real problem of non-additivity can arise. But one way round this is to make the plausible assumption that the gene effects in question are small: Fisher's microscope argument⁴ — that near to a good design only small changes are likely to be advantageous — suggests that, in the final stages of the perfection of a character, only genes of small effect matter.

Thus, for genes of small effect, additivity is restored and the correctness of Hamilton's rule is restored with it¹. (Population geneticists may be interested in genes of large effect, and it is perfectly reasonable to doubt if characters are anywhere near perfection, so not everyone will be happy with this assumption. Note that a gene can have a small effect through rarity of expression even if it has a large effect on the occasions it is expressed.)

The method used by Queller is Price's covariance selection mathematics, a powerful technique of general value¹ that was originated and expounded by Price in brief articles^{5,6}. For Queller, the value of

the technique was that it enabled him to change easily from counting genes in offspring according to the individual producing the offspring (the usual population genetics method of accounting by results), to counting according to the individual whose genotype caused the existence of the offspring (the inclusive fitness method of accounting by control).

In closing, I must mention a paper unjustly neglected by Queller, in which Price's method was first applied to inclusive fitness problems. This remains the most general and satisfying derivation and justification of inclusive fitness, at the same time as being remarkably simple. Queller cannot appeal to the obscurity of the journal in which the paper appeared, but he can plead for clemency on the grounds that its author himself later neglected the existence of his own strong and powerful result. Readers may have guessed that the author in question is W.D. Hamilton^{7,8}. His hitherto unsung primacy in providing a simple and rigorous justification for inclusive fitness, together with his well-recognized provision of the idea in the first place, inspire me to end close to where I began: Hamilton rules OK! □

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Developmental biology

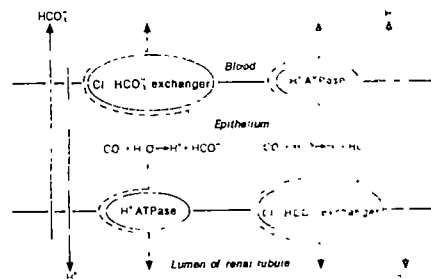
A reversible epithelium

from Jared M. Diamond

EPITHELIA are the sheets of cells that line body cavities such as the intestinal lumen. They are responsible for active transport of specific solutes, either from lumen to blood or vice versa depending on the particular solute and epithelium. In most cases the direction of transport is fixed, even though the rate of transport may be subject to physiological regulation. On page 368 of this issue G.J. Schwartz, J. Barasch and Q. Al-Awqati describe an unusual case of a reversible epithelium, which can transport the same solute in either direction in response to physiological need. The whole arrangement of pumps, exchangers and vesicles within the epithelial cell is reversible in detail. The tissue not only is a curious violation of the usual rules but also offers a promising system for learning how epithelia become programmed during development.

Schwartz *et al.* have studied the inter-

calated cells of the cortical collecting tubule (CCT) in the kidney. These regulate blood pH by either secreting H⁺ and absorbing HCO₃⁻, or secreting HCO₃⁻ and absorbing H⁺. The main physiological stimulus converting the tubule from net HCO₃⁻ secretion to net H⁺ secretion is the acid load. Schwartz *et al.* identify H⁺-secreting or HCO₃⁻-secreting cells by the endocytosis of fluorescent markers from the luminal or blood surface of the tubule, respectively. Since epithelial cells are commonly found to transport a given solute in a fixed direction, one might expect the CCT to contain two H⁺/HCO₃⁻-transporting cell types with opposite functions and the mitotic proliferation (or transport rate) of each cell type to respond to pH levels. Instead, mitotic figures are absent in intercalated cells of acid-loaded animals and the total number of cells remains virtually unchanged — 137 per mm



Depending on the respective locations of an ATPase and an exchanger, controlled by the acid load, the intercalated cell of the cortical collecting tubule either secretes H⁺ (left) or HCO₃⁻ (right).

of tubule after loading, compared with 129 per mm before loading. But the number of acid-secreting cells increases 10-fold, from 7 to 74, and the number of HCO₃⁻-secreting cells decreases correspondingly, from 122 to 63. This is evidence for a change in direction of transport in the same cell, rather than proliferation of a cell type with a certain direction of transport.

The change of direction is a result of the simple reversal of the arrangement of the transport mechanism, which consists of a proton-pump ATPase at one cell face and a Cl⁻/HCO₃⁻ exchanger at the opposite face (see figure). In H⁺-secreting cells the ATPase is at the luminal membrane and the exchanger at the blood-facing membrane; these locations are reversed in HCO₃⁻-secreting cells. The ATPase originates from intracellular vesicles which fuse with either the luminal or the blood-facing membrane, so that the ATPase is inserted into the appropriate membrane. Thus, one critical link in the control of transport direction by acid load is control of direction of vesicle movement.

These experiments open the way to exploring other steps in the chain of controls in the CCT. Insertion of the Cl⁻/HCO₃⁻ exchanger has not been studied and the detailed time course of acid control of ATPase insertion is not yet known. But this study also has broader significance for the developmental biology of epithelia in general. Biologists have long wondered how the genetic information in a radially symmetrical egg becomes translated into radially asymmetrical cells, tissues and animals. In epithelia, this question is posed in a particularly simple form, as functions are segregated between two cell faces. Membrane biologists have learned much about the details of the transport mechanism at each face but have so far paid little attention to developmental questions of how each mechanism gets inserted into the 'right' end of the cell. Having a cell in which the direction of insertion can be experimentally reversed may make it easier to answer questions such as these.

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Particle physics

How to detect solar neutrinos

from W. Hampel

EXPERIMENTS designed to observe the neutrinos produced in the fusion reactions in the interior of the Sun provide the only way to test the theories of energy generation in stars. Another motivation for solar-neutrino experiments comes from particle physics: they may be the only possible way to obtain information on neutrino mixing (oscillations), a question of great importance in Grand Unified Theories. This is because neutrino mixing could be observable only in experiments with very large source-detector distances. Two recent papers^{1,2} put forward a new concept for a solar-neutrino detector and evaluate its ability to detect neutrino oscillations.

Neutrino mixing occurs if neutrinos are superpositions of two or more mass eigenstates with different rest masses. This leads to oscillations of the electron neutrinos, ν_e , produced in the Sun with neutrinos of other flavours (muon- or tau-neutrinos, ν_μ or ν_τ) on their way from the Sun to the detector. In fact, the discrepancy between the theory and observation for the only solar-neutrino experiment carried out so far, the Brookhaven Chlorine Experiment³, may be due to neutrino oscillations. Whether this is true or not will be decided by the outcome of the Gallium Experiment⁴, the only other solar-neutrino detector that has had its feasibility demonstrated in a pilot experiment.

Since both chlorine and gallium detectors employ the radiochemical technique that is based on inverse β decay, they are sensitive only to ν_e and measure the integrated (above their respective energy thresholds) energy spectrum of solar neutrinos. Therefore, they give only partial information on neutrino oscillations (through a depleted rate compared to that expected without oscillations)⁵. To learn more about neutrino oscillations, it will be necessary to use a detector able to give direct information on the energy spectrum of solar neutrinos. The detector proposed by Cabrera *et al.*¹ has this feature. It is based on the elastic scattering of neutrinos from electrons. Figure 1a presents the spectrum of the proton-proton (pp), ^7Be and ^8B solar-neutrino sources (the other solar-neutrino sources are less important and have been omitted from this figure) along with the recoil electron spectra expected for these sources from the $\nu_e + e^-$ reaction. Solar-neutrino detectors based on this reaction have been proposed before (for example, refs 6 and 7). In these proposals, the detection of the recoil electrons is through their Cerenkov radiation in water. This method, however, is restricted to high energy (≥ 5 MeV) recoil electrons and thus only allows observation

of the ^8B neutrino component (see Fig. 1).

The detection method suggested by Cabrera *et al.* is completely different. It is based on observing the temperature change of a macroscopic silicon mass (of the order of 1 kg), when a recoil electron is produced through a neutrino interaction and deposits its energy in the silicon block. For instance, a 100 keV recoil electron would raise the temperature of 1 kg Si from 1 mK to about 4 mK. This principle,

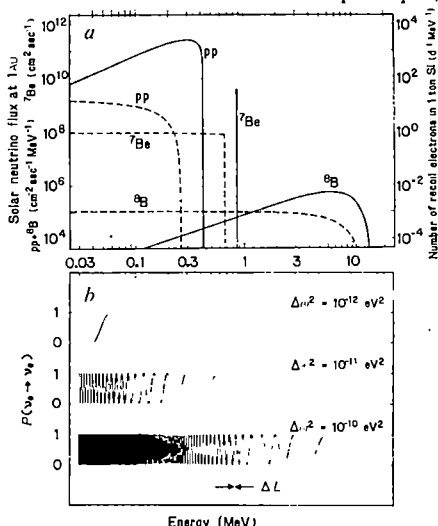


Fig. 1 a, Energy spectrum of the solar pp, ^7Be and ^8B neutrinos with the corresponding recoil electron spectra produced in the $\nu_e + e^-$ reaction. b, Probability $P(\nu_e \rightarrow \nu_e)$ of an electron neutrino ν_e emitted in the solar core reaching a terrestrial detector as ν_e . P as a function of neutrino energy is plotted for different Δm^2 .

the bolometric detection of solar neutrinos, was first investigated by Drukier and Stodolsky⁶ but their proposal was based on the coherent scattering of neutrinos from nuclei through the neutral current interaction and on a different technique (superconducting grain detector).

There are two major problems connected with bolometric detection of neutrinos. One is the problem of measuring small temperature changes in the mK region. The other is how to reduce the background to the very low level required for the detection of neutrinos. Concerning the temperature measurement, Cabrera *et al.* suggest, for instance, using superconducting thin film, such as tungsten deposited on the surface of the Si block. Determination of the resistance of this film would provide a measure of the temperature with a precision of about 10 keV in the energy range 10 keV–2 MeV.

Background is the more severe problem; the difficulties may be even larger with bolometric neutrino detection than with other types of detectors, since deposition of energy in the keV to MeV range is

not at all specific to neutrinos. All radiations, either from radioactive impurities in the Si itself or from outside sources (α -, β -, γ -radiation, neutrons, cosmic-ray particles), can dissipate energy in this interesting range and thus mimic a neutrino-scattering event.

As a first step, Cabrera *et al.* suggest constructing a prototype detector using a single Si block (~ 1 kg) and studying its properties with non-neutrino sources. Next, a larger detector (~ 100 kg) would be built for use in a reactor anti-neutrino experiment. Finally, a solar-neutrino experiment is planned with about 10 tons of Si ($\sim 10,000$ single cells).

Assuming such a detector could ever be built, what could be learnt from it about solar-neutrino oscillations? Restricting the discussion to the case where mixing of the ν_e occurs with only one neutrino of another flavour, the important quantity is the probability P that a ν_e emitted in the centre of the Sun is still (or again) a ν_e when it arrives at the detector:

$P(\nu_e \rightarrow \nu_e) = 1 - \sin^2 2\lambda \cdot \sin^2(\pi L \Delta m^2 / 2.48 E_\nu)$, where λ is a mixing angle ($0 < \lambda < \pi/4$), L is the distance between source and detector ($L = 1 \text{ AU} = 1.5 \times 10^{11} \text{ m}$), $\Delta m^2 = |m_1^2 - m_2^2|$ is the difference of the squared masses of the two involved neutrino mass eigenstates, in eV^2 , and E_ν is the neutrino energy in MeV. $P(\nu_e \rightarrow \nu_e)$ as a function of the neutrino energy is shown in Fig. 1b for maximal mixing ($\lambda = \pi/4$) and three different values of Δm^2 . Thus, the original solar-neutrino spectrum Φ (Fig. 1a) is modulated as a function of energy with P yielding ΦP as the ν_e spectrum incident at the detector site.

Neutrino oscillations affect the recoil electron spectrum produced by the $\nu + e$ reaction because the cross-section for ν_e is about six times larger than that for neutrinos of other flavours. This is because the neutral current (exchange of Z^0 bosons) contributes to the cross-sections for neutrinos of all flavours, whereas the charged current (exchange of W bosons) affects the $\nu_e + e^-$ reaction only. Thus, neutrino oscillations result in reaction rates that are depleted compared with the situation without oscillations. An advantage is that this comparison can be carried out separately for the different solar-neutrino sources (in contrast to the case for radiochemical detectors). This is especially useful for the pp neutrinos, since their reaction rate in the absence of neutrino mixing can be predicted rather reliably without use of detailed solar models. Measurement of the depletion in the pp reaction rate thus yields P averaged over the pp-neutrino spectrum.

The most important quantity to be determined is Δm^2 . As can be seen from Fig. 1, different values of Δm^2 affect the ν_e spectrum incident at the detector in different ways. For the continuum ν sources, this in turn has an impact on the shape of the resulting recoil electron spectra. Thus, careful examination of these spectra

should reveal Δm^2 and, combined with the information on P , the mixing angle λ .

Among other interesting effects discussed by Krauss and Wilczek² (such as the modification of vacuum oscillations by matter effects) there are two concerning the nearly monoenergetic ^7Be neutrinos. The first occurs because the distance L between the Sun and Earth varies by 3.4 per cent in the course of the year due to the eccentricity of the Earth's orbit. Trivially, this leads to a $1/L^2$ variation of the total solar-neutrino flux at the Earth. Because the equation for P depends on L , this variation also causes a periodic horizontal displacement of the P -curves in Fig. 1b, with an amplitude indicated in Fig. 1 by ΔL . Thus, the ^7Be recoil electron rate is expected to vary in time with a period and an amplitude depending on both Δm^2 and λ , an effect observable for $\Delta m^2 > 10^{-10} \text{ eV}^2$ and values of λ that are not too small.

The second effect is not related to neutrino oscillations. Since the electrons captured by ^7Be nuclei in the solar core have a Maxwellian velocity distribution depending on the local temperature T , the ν_e

emitted in this capture reaction have a spread in energy ($\sim 1 \text{ keV}$). Hence, the ^7Be recoil electron spectrum in Fig. 1 does not have a sharp cutoff at its endpoint energy. A measurement of the exact shape of this spectrum near the endpoint energy thus allows determination of T (requiring, of course, an energy resolution better than 10 keV).

Clearly, although the solar-neutrino detection suggested by Cabrera *et al.* and other detectors with similar capabilities in neutrino-energy spectroscopy are still far from being built, it would be well worthwhile investing in their development. \square

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Vertebrate palaeontology

First marsupial fossil from Asia

from Michael J. Benton

MARSUPIALS, pouched mammals like kangaroos and opossums, are today restricted to Australia and the Americas (Fig. 1). How this split distribution came about has been much debated by biogeographers. A northern dispersal route was favoured at first, with the early marsupials travelling from the Americas across Asia to Australia, but no evidence of marsupials had been found at that time in Asia. The acceptance of continental drift led most people to prefer a southern dispersal route from South America to Australia via Antarctica (Fig. 2). Now that evidence of a fossil marsupial has

been reported from central asiatic USSR¹, the question is again open.

The specimen is a single tooth, identified as a first or second upper molar from the right side of the jaw. It is very small (1.7 mm long by 1.6 mm across) and its closest affinities seem to be with the opossums (family Didelphidae, subfamily Didelphinae). It is most like a tooth of the European fossil opossums *Amphiperatherium* and *Peratherium*, and the North American *Herpetotherium*. The new Russian find comes from the middle of the Aksyr Formation, which is lowermost Oligocene (almost 37 Myr ago), in

the Zaysan territory in eastern Kazakhstan, central Asia.

The fossil record of marsupials was fairly simple until a few years ago. The oldest forms came from the late Cretaceous (84–65 Myr) deposits in North America, in which fossils are quite common. Up to 30 species of early marsupials have been identified in Alberta, Montana and Wyoming; these are divided into three families, two of which died out with the dinosaurs at the end of the Cretaceous period and the Didelphidae (opossums), which has survived to the present day. A few late Cretaceous marsupials have also been reported from Peru and Bolivia in South America.

In the subsequent Tertiary period, the didelphid marsupials spread to Europe, where they survived until the Miocene (about 20 Myr). In North America, the didelphids also become extinct in the Miocene but then reinvaded from South America about three million years ago. During the Tertiary, many new groups of marsupials arose in South America, including equivalents of sabre-toothed cats and some dog-like forms, but only three families still survive.

There are now 13 families of marsupials in Australia. The fossil record is sparse, with only a few odd teeth and jaws known from the late Oligocene, but there are more abundant and diverse remains from the Miocene onwards.

Three years ago, a South American marsupial (a polydolopid) was found in the Eocene deposits (about 40 Myr) of Antarctica², a find that seemed to support the theory of a southern dispersal route, with an ice-free Antarctica as the bridge between the southern tip of South America and Australia. Then, two years ago, marsupial remains in Africa were reported by two independent teams^{3,4}—but these are probably the remains of animals that wandered into Africa from Europe and thus do not help to disentangle the palaeobiogeography of early marsupial dispersal.

Although the Asian tooth may seem to provide support for the northern dispersal theory, it is approximately equivalent in age to the oldest known Australian marsupials, and so occurs too late to fit with the theory. It is also a didelphid, a European–American form, with no particular Australian affinities. Most probably the didelphids were at one time distributed more widely than has been thought and then died out without leaving any descendants in Europe, Africa and Asia. \square

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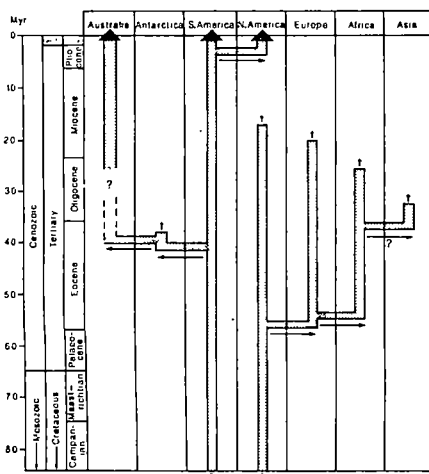


Fig. 1 Probable relationships between marsupial groups in different continents.



Fig. 2 The two possible dispersal routes with the positions of the continents as they were 80 Myr ago (modified from ref. 5).

Metamorphic geology

Tectonics of metamorphism

from Michael Brown

LARGE tracts of the continents of the Earth are formed by rocks which have undergone regional metamorphism — that is, their mineral assemblages indicate that they have been subjected to elevated temperatures and pressures at some time in their past. Typically, these areas are located in orogenic belts or Precambrian shields, and show an intimate relationship between metamorphism and tectonic deformation. Generally accepted tectonic settings for regional metamorphism are continental margins associated with subduction, such as the west coast of South America; island arcs, such as those of South-east Asia; and zones of continental collision, such as the Alps and Himalayas. But the Hercynian metamorphism of the Pyrenees does not fit easily into this framework, as it must be explained by a tectonic setting involving neither subduction nor continental collision. On page 330 of this issue¹, S.M. Wickham and E.R. Oxburgh use data from the Pyrenees to formulate a model of continental rifting, with or without strike-slip movement, as the setting for regional high-temperature metamorphism.

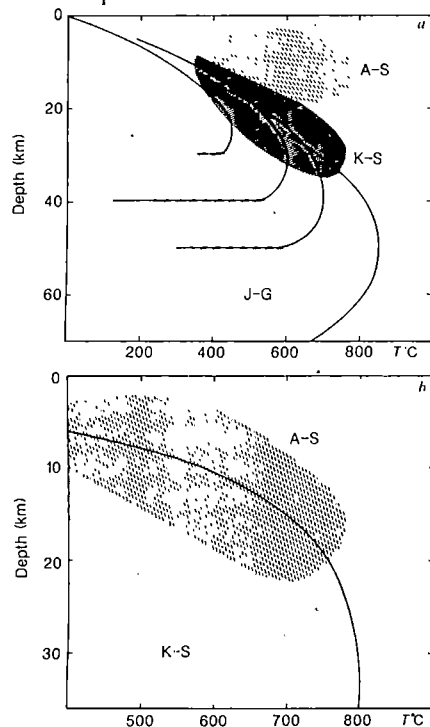


Fig. 1 *a*, P - T paths followed by rocks buried initially at 70, 50, 40 and 30 km after overthrusting (after Fig. 6c of ref. 6). A-S, andalusite-sillimanite facies series; K-S, kyanite-sillimanite facies series; and, J-G, jadeite-glaucophane facies series. *b*, Uplift P - T path followed by rocks initially metamorphosed at 800°C and at 35 km depth undergoing approximately adiabatic decompression; rate of uplift 2 mm per year (after model B of Fig. 9 of ref. 15). A-S and K-S as in *a*.

Metamorphic terrains commonly develop as paired belts, in which one member is characterized by blueschists and eclogites, which indicate low temperature and high pressure conditions and which is interpreted as having been developed at a site of low heat flow, such as a subduction zone; the other member is characterized by high-temperature metamorphism and the development of migmatites and anatectic granites, and is interpreted as having been developed at a site of high heat flow, such as that beneath an associated volcanic arc. In detail, regional metamorphic terrains have been classified by their mineral assemblages into facies series types such as andalusite-sillimanite, kyanite-sillimanite and jadeite-glaucophane. It is implicit in this classification that an entire metamorphic terrain can be described by a single geothermal gradient and that higher-grade mineral assemblages develop from lower grade ones similar to those now found along the present erosion surface. This traditional view has been replaced by one in which individual rocks¹ and minerals² can be used to derive paths in pressure-temperature (P - T) space, which can be related to the tectonic setting^{3,6}. The loci of peak or slightly post-peak P - T conditions as preserved by the mineral assemblages — the metamorphic geotherm — results from the intersection of P - T paths for individual rocks with the erosion surface. It is this which is represented by the particular facies series.

Assuming that a uniformitarian approach to the problem is valid, we can try to unravel the tectonic history of metamorphic terrains by relating the characteristic features of young metamorphic belts to their tectonic settings. In essence, this means establishing the characteristic P - T paths of metamorphism in different plate-tectonic settings. For instance, the jadeite-glaucophane facies series can be generated by subduction. However, the Pyrenees comprise a series of structural and thermal domes with condensed metamorphic zonal sequences of the andalusite-sillimanite facies series, culminating in migmatites (rocks that have been partially melted) and abnormally high crustal temperatures and hence thermal gradients. It is this type of metamorphism that Whickham and Oxburgh explained.

Pioneering work on metamorphism associated with continental rifting and metamorphism due to magmatic accretion⁷ did not lead to a level of understanding of the thermal structure associated with such metamorphism comparable to our knowledge of overthrust terrains^{2,6}, although both are potentially important in

the island arc and active continental margin settings which so dominate lithospheric plate margin interactions. It is in this latter context that the work of Wickham and Oxburgh¹ is important.

Although it has been developed for a metamorphic terrain that cannot have formed at a subducting margin, the model has implications for high-temperature metamorphism in general, as extension and strike-slip movements are common in island arcs and active continental margins⁹. However, Precambrian meta-



Fig. 2 Typical texture produced by overstepping of reactions during approximately adiabatic decompression. In this case, garnet (black) plus quartz (medium grey in centre of view) reacts to orthopyroxene (granular mineral surrounding quartz crystal) plus cordierite (white to grey mineral in central part of view clearly replacing garnet); garnet-orthopyroxene-cordierite gneiss from the Sharyzhalgay Complex, USSR. Long dimension of photomicrograph is 5.5 mm, crossed polarized light.

morphic terrains such as the Napier Complex of Antarctica and the Arunta Complex of Australia, which have suffered extreme temperatures (900–950°C) and are characterized by approximately isobaric cooling to more moderate temperatures (600–650°C) during substantial periods of time, do not fit into a uniformitarian model of metamorphism based on plate tectonics theory. Moreover, the role of magma in regional metamorphism is poorly understood, although examples have been described from Greenland¹⁰ and Australia¹¹.

During continental collision, such as in the Alps, regional metamorphism may be related to crustal thickening, either by overthrusting or by uniform strain. The result is a characteristic anticlockwise loop in depth-temperature space (Fig. 1*a*), with one third or more of the P - T path being within 50°C of the maximum temperature achieved during burial and uplift⁶, and high-pressure metamorphism followed by increasing temperature and decreasing pressure.

More difficult to relate to tectonic setting with certainty are those terrains characterized by overstepping of reactions during decompression^{12–14} (Figure 2 illustrates an intermediate stage of a reaction). It is in the context of these metamorphic terrains characterized by approximately adiabatic decompression (Fig. 1*b*), that

the model for high-temperature metamorphism proposed by Wickham and Oxburgh¹ for the Pyrenees is important. Other essential features of the Pyrenean belt are the contemporaneity of sedimentation and metamorphism and the post-metamorphic development of granitoids in the lower crust. Such features are common in other high-temperature metamorphic belts such as British Columbia¹² and southern Brittany¹³, as well as in those found in Japan. The suggestion of Wickham and Oxburgh that the high-temperature member of paired metamorphic belts might develop by rifting and extension in island arcs is plausible and attractive. It is also timely, coming before a Royal Society meeting (January 1986) on Tectonic Settings of Regional Metamorphism. □

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Human genetics

Measuring mutation in man

from Mary F. Lyon

GENETIC disease is an important cause of ill health and of physical and mental handicap, particularly in childhood, and results in a significant number of premature deaths. Major advances in preventing the birth of genetically affected children are being made, through genetic counselling, prenatal diagnosis and the use of recombinant DNA methods. But mutation remains a problem, resulting in the levels of many genetic diseases being maintained despite adverse selection, and it is becoming increasingly important to assess the possible increase in mutation rates caused by chemical or physical agents in the environment. At two recent meetings* plans were put forward for an international collaboration to measure the mutation rates in man by studying the offspring of cancer patients who have survived to reproduce after radiotherapy and chemotherapy. This should, for the first time, provide information on the effect of increased mutation rates on the incidence of hereditary diseases in man.

Genetic diseases may cause death before reproductive age, like Duchenne's muscular dystrophy, or result in failure to reproduce through, for example, severe mental defect due to fragile-X syndrome. Such diseases are maintained at their present frequency by a balance between fresh mutation and adverse selection. New mutations mean that affected individuals occur without any previous history of the disease in a family, making prenatal diagnosis of limited usefulness. Intensive efforts to clone the gene for Duchenne's muscular dystrophy were discussed recently in *Nature*^{1,2} and, when successful, will make it possible to prevent the birth of

affected sons to known carrier mothers. But one third of all cases are due to new mutation and the means of preventing these is unclear³, as it would be impractical to screen the whole population.

If the mutation rate rises, the incidence of such mutationally maintained diseases will rise, so efforts are being directed towards the detection of mutagens in the environment and their control or elimination. The International Commission for Protection against Environmental Mutagens and Carcinogens (ICPEMC) aims to evaluate scientific data relating to environmental mutagens and carcinogens and to provide information for estab-

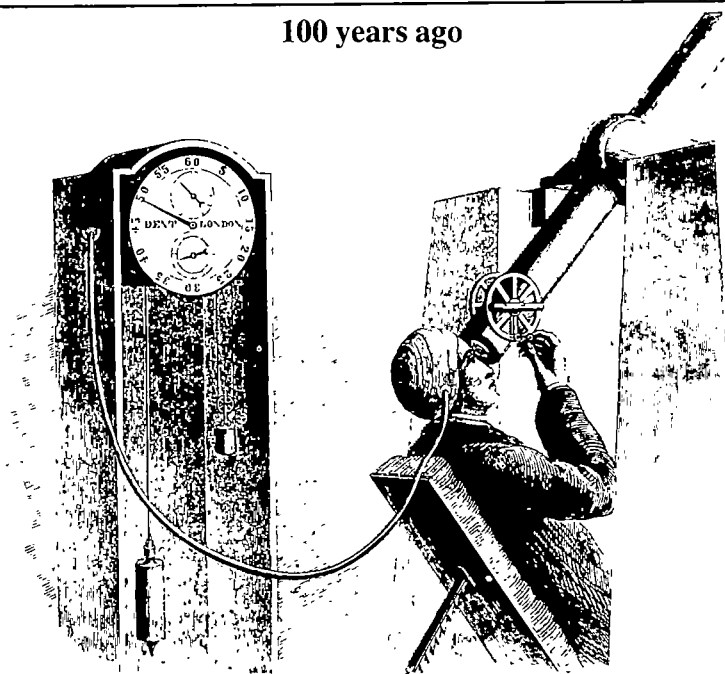
lishing guidelines for regulatory or legislative action.

The question of detection and protection against germ-cell mutagens in man is highly complex. So far, successful methods of detecting changes in mutation rate directly in human populations have not been found⁴. Extrapolation from experiments is thus necessary, but this is only possible with data obtained in mammalian germ cells, because there are wide variations in the sensitivity of different cell types, which are not understood. Germ-cell data are laborious and costly to obtain and so are not available for many agents.

Relatively extensive information for several cytotoxic drugs that are used in cancer chemotherapy or as immunosuppressants does, however, indicate that some of these agents, for example cyclophosphamide⁵, are strongly mutagenic for certain germ-cell stages. With the recent improvements in the treatment of cancer occurring in children and young adults, many survivors of cancer treatment are living to reproduce. For the sake of the patients and their children there is a clinical need to know the long-term prospects, not only about the likelihood of second cancers in the patients but also about their fertility and the normality of their children.

Investigations into the reproductive histories of surviving cancer patients have already begun and preliminary results were presented at the first meeting. Examples include surveys of childhood cancer in Italy (G. Pastore, Turin), the United Kingdom (G. Draper, Oxford) and the United States (J. Mulvihill, Bethesda); of testicular cancer in Norway (S. Fossa, Oslo) and the United Kingdom, and of Hodgkin's disease in the United States (S. Donaldson, Stanford). In the

100 years ago



The transit instrument of the Bedford Observatory, with observing chair and clock. From *Nature* **33** 59, 19 November 1885.

*Workshop on possible genetic effects in offspring of surviving cancer patients, Oslo, May 3-5, 1985 (sponsored by ICPEMC and the Norwegian Cancer Society). Follow-up meeting, Lyon, 1-3 October, 1985 (sponsored by IARC).

course of the meeting, it became clear that it would be very difficult for surveys in a single country to recruit sufficient patients and offspring to obtain meaningful results. Extrapolations from animal data suggest that many thousand offspring of patients treated with cytotoxic drugs will have to be studied to detect an increase in genetic disease resulting from such treatment⁶. Examination of survivors from atomic bomb explosions have also shown that large numbers are needed to detect the effects of radiation⁴.

At the first meeting, therefore, an international collaborative study was suggested, using a standardized protocol so that results could be pooled. A smaller group, including representatives of the Union Internationale contre Cancer, later met to formulate practical arrangements for initiating the study. The types of cancers to be studied would include childhood cancers, testicular tumours, and Hodgkin's and other lymphomas. Transplant patients and others treated with immunosuppressive drugs known to be mutagenic, such as cyclophosphamide^{7,8}, might also be included. Records would be kept of fertility, outcome of pregnancy including spontaneous abortions, birth weight, malformations and cancer in the offspring. Samples of blood would also be obtained from the patient, the other parent and the offspring, and stored in the hope that studies of chromosomes, protein (K. Berg, Oslo) or DNA variants would prove possi-

ble — potentially these could yield much more detailed information than records of clinical abnormalities⁹. At present, however, such studies are much more laborious and expensive; the DNA studies, though potentially the most informative, are also the most expensive (P. Pearson, Leiden).

Such a programme would benefit the patients and their offspring by providing information on the levels of genetic risk incurred from ever more aggressive cancer treatments. It is possible that the relative mutagenicity of different treatments could be assessed, so that the genetic risk could be reduced. Scientifically, the results could be compared with the extrapolations already made from animal work⁶ to assess the validity of such extrapolation. Most important, data would become available on the effects that known doses of strong mutagens have on germ-cell mutation in man. This will be a major step in tackling the problem of mutation as a cause of human genetic disease. □

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Geophysics

International Geomagnetic Reference Field revision

from David R. Barraclough

THE International Geomagnetic Reference Field (IGRF) is a series of mathematical models of the main geomagnetic field and its secular variation. The models consist of sets of spherical harmonic (or Gauss) coefficients. IGRF has become widely used for deriving values of geomagnetic components used in, for example, studies of magnetic anomalies and investigations of charged particle motions in the ionosphere and magnetosphere.

Since it was first adopted by the International Association of Geomagnetism and Aeronomy (IAGA) in 1968 (IGRF 1965, ref. 1), IGRF has been revised three times: IGRF 1975 (ref. 2); IGRF 1980 (ref. 3); and now IGRF 1985. Details of the derivation of the original IGRF and of its development up to 1981 have been given by Zmuda⁴ and Peddie⁵.

The latest revision of IGRF was considered by Working Group 1* (analysis

of the main field and secular variations) of IAGA Division 1 during the Fifth General Assembly of IAGA held in Prague in August 1985. The following additions and modifications to IGRF 1980 were recommended.

- The extension of the definitive international geomagnetic reference field (DGRF) to 1980.0 by the adoption of a new model (DGRF 1980) to replace IGRF 1980.
- The addition of an international geomagnetic reference field for the interval 1985.0 to 1990.0 (IGRF 1985) consisting of a model of the main field at 1985.0 and a predictive model of the secular variation for use in adjusting the main-field model to dates between 1985.0 and 1990.0.
- The adoption of a provisional international geomagnetic reference field for the interval 1980.0 to 1985.0 (PGRF 1980), defined by linear interpolation between the coefficients of DGRF 1980 and IGRF 1985 (main field).
- The addition of a series of main-field

models for the epochs 1945.0, 1950.0, 1955.0 and 1960.0 (IGRF 1945, IGRF 1950, IGRF 1955 and IGRF 1960).

DGRF now spans the interval 1965.0 to 1980.0 with four main-field models for 1965.0, 1970.0, 1975.0 and 1980.0 (DGRF 1965, etc.). For dates between the epochs of the models, linear interpolation between the coefficients of the two models on either side of the date is to be used. A similar procedure is to be used for dates in the interval 1945.0 to 1965.0, using the IGRF 1945, IGRF 1950, IGRF 1955, IGRF 1960 and DGRF 1965 models, as appropriate. Extrapolation back to 1940.0 will probably be reasonably accurate, though this was not formally recommended by the working group.

Further revision of DGRF is not anticipated. The pre-1965 models (IGRF 1945 to IGRF 1960) will probably be replaced by definitive models in 1987. The newly adopted DGRF 1980 model replaces the former PGRF 1975 and IGRF 1980. The present PGRF 1980 will be superseded when a definitive model of the main-field at 1985.0, different from IGRF 1985, is adopted.

The main-field models for 1960 to 1985 have 120 coefficients each and extend to degree and order 10. The main-field models for 1945 to 1955 and the predictive secular-variation model have 80 coefficients and extend to degree and order 8. The coefficients are given in the Schmidt quasi-normalized form⁶ and refer to a sphere of radius 6,371.2 km. When converting between geodetic and geocentric coordinates the use of the international atomic unit (IAU) ellipsoid⁷ is recommended; this ellipsoid has an equatorial radius of 6,378.16 km and a flattening of 1/298.25.

The coefficients of the IGRF models and computer programs for synthesizing field values are available from:

- World Digital Data Centre C1, British Geological Survey, Murchison House, West Mains Road, Edinburgh EH9 3LA, UK.
- World Data Center A, National Oceanic and Atmospheric Administration, EDIS/NGSDC (D62), 325 Broadway, Boulder, Colorado 80303, USA.
- World Data Center A for Rockets and Satellites, Code 601, NASA/Goddard Space Flight Center, Greenbelt, Maryland 20771, USA. □

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Meteorite impacts on humans and on buildings

SIR—From nine years of observation with the Meteorite Observation and Recovery Project, a network of 60 cameras in western Canada, we have derived the frequency of meteorite falls on the Earth as a function of the total mass of meteorites to reach the ground for each event¹. Assuming that the total mass of an event is twice the mass of the largest fragment observed by the cameras, we conclude that

$$\log N = -0.689 \log m + 2.967$$

where N is the number of events per year in which a mass of at least m grams of meteorite is deposited in an area of 10^6 km^2 . This formula predicts 39 events per year with a mass of at least 100g in each million km^2 , or 5,800 such events on the total land area of the Earth.

Our present concern is to estimate the probability of persons or buildings being struck by meteorites. Our major assumptions are: (1) A human being occupies an area of 0.2 m^2 . (2) The smallest impact likely to be reported would involve a fragment of a few grams. (3) Typical meteorite falls consist of five major fragments. (4) Meteorite fragments larger than 200 g will normally penetrate a roof and ceiling. (We also assume that, if the total mass exceeds 500 g, each of the five fragments could penetrate a roof, whereas none of the pieces from lesser events will do so.) (5) Residents of North America spend 5 per cent of each day outside and 95 per cent of the day protected by a roof. (6) The total roof area of buildings averages 50 m^2 per member of population.

On the basis of these plausible assumptions we derive the frequency of impacts on people and on buildings and we wish to compare the values with recent experience. For this purpose we consider the population of the United States plus Canada (about 2.5×10^8 persons) since we expect news coverage of relatively minor events to be more complete for this sample than in many densely populated areas of the world. For this North American sample, our assumptions predict an annual rate of 0.0055 impacts on people (one event per 180 years) and 0.80 impacts per year causing damage to buildings.

The only documented case of a person being struck by a meteorite appears to be a fall on 30 November 1954². A fragment of a stony meteorite weighing 3.9 kg penetrated the roof and ceiling of a house in Sylacauga, Alabama, bounced off a large radio and struck a woman who was asleep on a couch, inflicting painful bruises. At first glance it would appear unlikely that there would be even one known event only 31 years ago, but the fact that there are no other verified cases elsewhere in the world indicates that impacts on people are extremely rare.

The prediction for impacts on buildings is more readily verified because the pre-

dicted rate is much higher. All known meteorite falls and chance recoveries are reported in the *Meteoritical Bulletin*, published about once per year in the journal *Meteoritics*. During the past 20 years, there are reports of 16 recoveries from fresh meteorite falls in the United States and Canada. For seven of these events, there was appreciable damage to some building, usually the roof of a home or garage. In the Louisville, Kentucky fall in January 1977, three separate buildings were struck, so there are nine reports of damage. Two other events, which we have not included, involved a small meteorite which caused no damage to a roof and a 1.3 kg object that damaged a mailbox. It is clear that impact on a building greatly increases the probability of recovery of the meteorite, since only a very small fraction of all falls will strike buildings, whereas half of the recent recoveries have such an involvement.

We would predict 16 damaged buildings in 20 years for this sample, which is close to the nine reports of damage. Since we would not expect small meteorites to be located, identified and reported in all cases, especially for minor damage to commercial buildings where there might be little interest in finding the cause of a leaky roof, we suppose the actual rate may be somewhat higher than our estimate. Our guess of five effective fragments per event may be too low.

To extrapolate to the entire world population, estimated at 5,000 million, then the number of events will increase by a factor of 20 over the North American sample if we use the same assumptions. One would then expect a person to be struck by a meteorite once in nine years and that sixteen buildings per year would receive some damage from meteorites.

We thank Dr Paul A. Feldman of the Herzberg Institute for valuable discussions.

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A question of cellular immortality

SIR—In "Human T-lymphotropic retroviruses" (*Nature* **316**, 395-403; 1985), as well as other articles many authors now speak of the "immortalization" of cells by the expression of oncogenes. This term is generally used to refer to the ability of a cell line to grow *in vitro* indefinitely, without senescence or a growth crisis. The use of the term, to those not versed in the minutiae of oncogene nomenclature, would seem to imply specific gene(s) whose func-

tion is to prevent cell senescence. This gene would seem to have no corresponding physiological function *in vivo*, however.

A more plausible explanation would be that the cell, at a certain point in its step-wise differentiation, fails to turn off a regulatory gene. The cell continues to express this regulatory gene and the genes whose expression it controls. The cell is "stuck" in a state of expressing inappropriate genes and is prevented from terminal differentiation. This observation would fit with the fact that established cell lines, while not necessarily tumorigenic, require fewer steps to render them tumorigenic, perhaps as little as a growth factor with a specific effect on a certain differentiation state.

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Weak violation — a new concept in relativity?

SIR—Quoting from *Nature News and Views*, "If there are violations of special relativity, they are at most weak violations, departures from what is not strict orthodoxy and not flat contradictions of it". Not everyone would agree with this statement. For many decades now, Einstein's relativity, and the need for absolute conformity with its basic tenets, have stood firmly in the way of any theoretical advance which dares to imply the slightest violation. But though it is improbable that we will ever see support for Marinov's claim, that the Earth's cosmic motion through space can be detected by tests confined to the laboratory, should this be demonstrated as proven, then Einstein's theory is violated by a death blow struck into its very heart.

Consistent with empirical data, a number of alternative laws of electrodynamics were shown by Clerk Maxwell to be derivable from a formula based on the relative velocity of interacting charges, taken in conjunction with what we would, today, term a "quantum electrodynamic" proposition (Fechner's hypothesis). Einstein's constraint, that any such law must satisfy the test of covariance, excludes a form of law which has recently been supported by experimental evidence from anomalous forces accompanying electric discharges in electrolytic solutions¹.

Consequently, research into practical applications of the indicated law has, at least until the present time, been retarded, blocked by the "inviolable" principle of relativity, and for the very reason that a successful outcome would be a strong violation and therefore improbable.

Whereas Marinov has sought to chal-

lenge Einstein directly on the speed of light issue, the Achilles' heel may already have been exposed in the application of electrodynamic law to electrochemical processes. Relativity, in its classical sense, will undoubtedly survive, but the real question is whether the test of time will continue to support Einstein's requirement that each and every relatively-moving observer constitutes his own personal reference frame for the locally-applicable laws of physics.

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Structure and activity of barbiturates

SIR—It has long been a puzzle that caffeine and other purines should be stimulants while the chemically similar barbiturates are sedatives¹. I suggest that the latter are no longer planar in solution, being hydrated to the *gem*-diols and having the molecule folded about its principal axis (boat configuration).

Hydration of the central carbonyl can reasonably be expected because of the high positive charge on each of the neighbouring amide nitrogens. The result of electron-withdrawal near a carbonyl group is most familiar as chloral hydrate. Such *gem*-diols are known to catalyse the hydration of carbon dioxide, if the pH is high enough for partial ionization of the diol². Most barbiturates would then be fully ionized as amides, unless they were substituted at both nitrogens. Veronal, therefore, should be inactive, and etorbarbital active, in promoting the hydration of dissolved carbon dioxide to carbonate.

As for biological activity, *gem*-diols are ideally constituted to bind the ionized carboxyl of GABA and other amino acids.

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Linking impacts and plant extinctions

SIR—The discovery of shock-metamorphosed quartz grains¹, in addition to earlier evidence from siderophile enhancements² and microtektite-like spheroids³⁻⁵ appears to confirm the existence of a bolide event at the Cretaceous-Tertiary (K/T) boundary, but a causal connection between this event and the extinctions of many plants and animals remains in dispute. The pattern of extinctions in the marine realm⁶⁻¹⁰ generally conforms to

the hypothesized effects of an asteroid impact, but evidence from the terrestrial biota has been somewhat paradoxical. Though the situation for land animals remains equivocal (because some of their fossils are suspected to have been reworked), recent evidence collected from both fossil pollen and palaeosols appears to support the marine data for a sudden, catastrophic change at the K/T boundary.

Latest Cretaceous pollen sequences recovered from many sites in Montana^{11,12}, Colorado¹³ and New Mexico¹³ display dramatic shifts in the ratio of fern spores to angiosperm pollen at the K/T boundary. These widespread palynofloral disruptions suggest that the terrestrial flora suffered a profound ecological shock at the boundary, and the close proximity of these pollen breaks to Ir anomalies and highly shocked quartz grains implicate an asteroid impact. The pattern of plant destruction and renewal is similar to that observed at Tunguska¹⁴, Krakatau¹² and Mount St Helens¹⁵. Initial devastation might have been produced by the shockwave¹⁶ that would have resulted from a terrestrial impact¹⁷.

In addition, information collected from palaeosols (M.D.S. and G.J.R., unpublished data) in Montana tends to support the pollen evidence for a change in the flora at the K/T boundary. Palaeosols from the Hell Creek Formation are well developed, weakly calcareous and have a microstructure indicative of warm, oxidizing conditions. These Cretaceous palaeosols combined with pollen data are suggestive of a well drained closed-canopy forest of subtropical aspect, dominated by broadleaf angiosperms. Mean annual rainfall was subhumid, but periodic, possibly monsoonal. The presence of mummified dinosaur corpses, as well as "growth rings" in the teeth of some endothermic vertebrates¹⁸, provides evidence for seasonally dry conditions. By contrast the palaeosols of the Tullock formation have a weak microstructure, and tend to be poorly developed with thick organic layers and abundant siderite nodules, suggestive of a cooler climate and poor drainage. In combination with pollen data these palaeosols are indicative of a temperate flora dominated by conifers and situated in a swamp environment. A modern analogue would be bald cypress swamps that presently exist over portions of southeastern North America. Coals are thicker and better developed in the Tullock formations, consistent with the hypothesized existence of poorly drained, widespread wetlands.

The change in palaeosols is abrupt and occurs directly above the pollen break at the K/T boundary, although it is difficult to say how sudden the palaeosol change was because of a complex erosional hiatus at the boundary. The palaeosol change is generally consistent with an interpretation made by Smit and van der Kaars¹⁹ for palaeochannels in the Bug Creek area.

Evidence from both pollen and palaeosols suggest a change across the boundary to unstable conditions in the early Palaeocene, shown by channel downcutting and followed by the establishment of a new geomorphic equilibrium. Long-term palaeosol and pollen changes are compatible with base-level adjustments, possibly related to a drop, then a rise in sea level. The synchronous occurrence of an Ir anomaly, spheroids, pollen breaks and shocked quartz at the boundary is suggestive of an additional catastrophic destabilization.

The presence of abundant palaeosols places constraints on the degree of resorting of fossils within the Hell Creek Formation. For example, it has been suggested²⁰ that nearly all fossil material contained in the Hell Creek faunal-facies was reworked and transported. This is an overstatement, as palaeosols would be destroyed in frequently disturbed environments, and the widespread occurrence of palaeosols in association with large fossils (less commonly with microfossils) suggests that much, if not most of this facies was formed in place. The truncation and strong development of palaeosols at the K/T boundary, together with the lack of palaeosols associated with fossils of the Bug Creek faunal-facies is consistent with the suggestion¹⁹ that some of these fossils were transported.

A more detailed picture of the Hell Creek-Tullock palaeoecological transition awaits further analysis of data. We believe, however, that at the moment the asteroid impact hypothesis provides the most parsimonious explanation for changes in plant communities across the K/T boundary.

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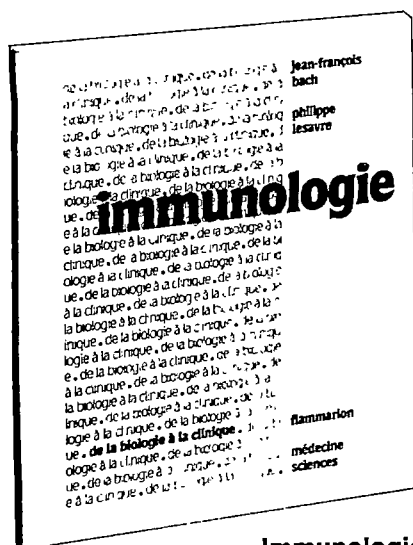
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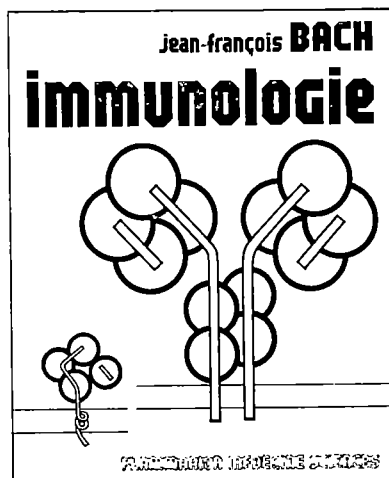
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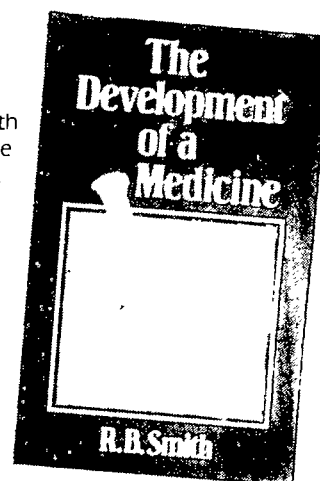
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Fall out over atomic tests

John Simpson

Fields of Thunder: Testing Britain's Bomb. By D. Blakeway and S. Lloyd-Roberts. Allen & Unwin:1985. Pp.243. Hbk £10.95; pbk £3.95.

Clouds of Deceit. By J. Smith. Faber & Faber:1985. Pp.174. Hbk £8.95; pbk £4.95.

A History of British Atomic Tests in Australia. By J.L. Symonds. Australian Government Publishing Service:1985. Pp.593. A\$28.

Just Testing. By D. Robinson. Collins/Harvill:1985. Pp.595. pbk £5.95.

In January 1985 a unique event occurred. A foreign judicial commission came to Britain to cross-examine current and former scientific civil servants about events thirty years ago classified as military secrets, but now released. The investigations of this Australian Royal Commission into the short and long term consequences of British nuclear testing in Australia took place at a time of growing concern that participants in nuclear weapon tests in the atmosphere were more susceptible in later life to cancer, and certain other diseases, than the rest of the population. This concern was heightened by the attempts of those who had fallen ill to gain compensation for their suffering, disabilities and possible premature death from their parent governments; the refusal of certain of those governments to admit liability, and the organization of pressure groups to assemble evidence to demonstrate liability and to lobby for changes in compensation rules. It was against this background, including the publication next month in Australia of the Report of the Royal Commission, that these four books were written and can be evaluated.

Several obvious distinctions can be made between the books. Symonds was commissioned to write by the Australian Department of Resources and Energy as a person who held an official position in the Australian Atomic Energy Organization, and had privileged access to Australian and British documents whereas the other three are written by professional journalists and writers. It should therefore be no surprise that the theme of Symonds' work is that no unexpected events or irresponsible actions of states, scientists or officials occurred and, equally unsurprising, that the other three books appear to start from the assumption, fostered by long standing governmental secrecy, that there are human and state culpability and errors to be exposed when investigating British nuclear testing activities. Symonds' implicit conclusion is thus that there is little to be concerned about as a consequence of the tests, whereas the overt conclusion of the other three writers is the exact opposite.

There are other differences in the coverage. Smith and Symonds only cover events in Australia, while Blakeway/Lloyd-Roberts and Robinson deal with all

the British atmospheric nuclear tests, including those at Christmas Island. Symonds writes a simple chronology of events, using only government documents, while Blakeway/Lloyd-Roberts and Smith use historical sections, based upon Royal Commission documents and hearings, interviews with people involved in the tests and newspaper reports to argue a particular thesis.

Blakeway/Lloyd-Roberts argue that governments should now acknowledge that some, though not all, test veterans suffered as a consequence of the tests, whereas Smith presents a pot-pourri of accusations that the British media were subservient to the government of the day (an unsurprising view given her personal difficulties in having stories on nuclear testing published in the *Sunday Times*) and the need for nuclear disarmament and support for the test veterans against their parent governments. By contrast, half of Robinson's book consists of interview material gathered from servicemen involved in the tests. He attempts to view events from the perspective of the men rather than their commanders.

The literary style and presentation used in the volumes also differs appreciably. Symonds uses camera-ready copy, has appendices after most chapters in which important documents are reproduced and has a curious system of references to the side of the text. There are also elements of

repetition in the text. It is thus essentially an extended, detailed and exhaustive report, rather than a commercially produced book and its structure does not make for easy reading. Yet as a work of reference, it contains much more documented information than any of the other volumes.

Robinson, in half his book, uses an impressive technique of short quotations drawn from interviews with people who participated in the tests juxtaposed either on the basis of topics (for example eating facilities on Christmas Island) or experiences of specific tests. These quotations are interspersed with brief medical histories of each new individual introduced into the book. The technique gives a sense of what it was like to be there, of the ignorance of what was happening felt by those who were taking part and the way in which rumours about events spread.

The Blakeway/Lloyd-Roberts work is the more polished of the other two books, offering both greater detail and wider coverage than Smith. It is also the only one of the four with an index. It contains a number of glaring factual errors, however (e.g. on page 153 a bomb is dropped from an aircraft at 13,000 feet and explodes at 15,000 feet) and also is guilty of misrepresenting the proposals made in the mid-1950s to hold tests of initiator devices for nuclear weapons in Scotland. Thus on page 159 in a passage about the Christmas Island hydrogen weapon tests we are told that "the decision was bred from the same scientific confidence and political expediency that produced the idea that tests should take place over mainland Britain" yet the massive difference in the nature of the two types of tests is conveniently ignored.

In addition, when these authors find themselves confronted by lack of information, they occasionally resort to what can only be described as smear tactics. For example (page 177) "there is no indisputable evidence that large numbers of men



Britain's second atomic test at Woomera rocket base in Australia in January 1954 — watched here by Sir William Penny who led the team carrying out the tests.

at Christmas Island were unknowingly exposed to radiation" yet later in the same paragraph we are told "the hydrogen bomb tests were administered and carried out by the same authorities responsible for the Australian tests, and there is no reason to believe that the safety precautions changed for the better once the move had been made to the central Pacific". Similar remarks, however, could be made about all the books other than that of Symonds on the grounds that none of them has accurately reproduced the listing of UK atmospheric tests and their yields published by the Ministry of Defence in 1984.

Because three of the books draw on much the same body of information for their accounts of events, the main differences are in their perspective upon and interpretation of history, rather than any revelations about what happened. Yet a number of issues of substance do emerge. One is the crude techniques used for specifying the requirements for the initial test sites (the use of a map to see how far the nearest large town was from the US Nevada site and adding a safety margin). Another was the error of exploding the first device in the 1953 test series at a time when the meteorological conditions were unsuitable and the disputed consequences of this for both aborigines and the aircrew sent to tackle the resultant nuclear cloud.

Further issues include the degree to which procedures laid down for radiation safety were actually complied with; the extent to which aboriginal intrusions into the Maralinga test site went unreported in official documents; the degree to which unjustified risks of exposing personnel to radiation were taken in pushing the British nuclear weapon programme through to fruition in six years and finally, there was the impact of war-time agreements with Canada and the United States on the relationship between the United Kingdom and Australia.

One unresolved issue remains central to all these works: what assumptions can be made about the relationship between exposure to nuclear radiation, especially at low levels, and ill health and premature death. The official view remains that threshold values can be established to distinguish between those who are "safe" and those at risk. Opponents argue against this, largely on the grounds of statistical correlations. The neutral reader is likely to remain unconvinced either way on this issue by reading any of these books, but they will no doubt provide additional ammunition for a controversy with profound human, scientific and political dimensions which seems likely to continue indefinitely. □

John Simpson is Senior Lecturer in the Department of Politics, University of Southampton, Southampton SO9 5NH, UK. A second edition of his book, The Independent Nuclear State: The United States, Britain and the Military Atom, will be published next year by Macmillan, London.

The ups and downs of an antibiotic

Trevor I. Williams

Penicillin: Meeting the Challenge. By Gladys L. Hobby. Yale University Press: 1985. Pp. 319. \$30, £30.

THE timing of the events surrounding the appearance of penicillin is well-established. It was discovered by Alexander Fleming in 1928 and the crucial research demonstrating its remarkable therapeutic potential was carried out by Howard Florey, Ernst Chain, and a small group of colleagues at Oxford between 1939 and 1941, with large-scale production being established in the United States by 1944. In 1945 Fleming, Florey and Chain shared the Nobel Prize for Physiology or Medicine. Although there remained much to be learnt about penicillin, the peak effectively was passed forty years ago. It is surprising, therefore, that interest in the history of penicillin has lately intensified and still remains controversial. Since 1979 there have appeared an important new biography of Fleming; two official biographies of Florey; and an official biography of Chain is shortly to be published.

It might be supposed, therefore, that there would be little room for yet further work on the history of penicillin. This book by Gladys Hobby is different, however, in that it is a comprehensive and well documented review of penicillin as a drug and thus largely avoids the problems of personality that are likely to beset biographers.

The treatment is not wholly objective, however and, like others before her, Dr Hobby explores the problem of why Fleming not only failed to develop penicillin himself but quickly lost interest in it. She is surely right in supporting the view that a change in the climate of medical opinion was a significant factor. In 1928, there was much scepticism about the potential of chemotherapy, especially in St Mary's Hospital, which was strongly orientated towards vaccine therapy under Almroth Wright. By 1939, however, the demonstrable success of the sulphonamides had changed the attitudes of many people very considerably.

In commenting on Fleming's own plea that he failed through lack of technical support she makes one very curious statement. She attributes to Chain a comment "that without partition and paper chromatography, infrared and ultraviolet spectroscopy, nuclear magnetic resonance and electronic spin resonance — techniques not known in the 1920s and early 1930s — isolation of an unstable natural product such as penicillin would have been exceedingly difficult". It is hard to believe that Chain ever made such a statement,

for none of these techniques played any part in the original purification of penicillin for clinical trial. A possible explanation is that the author is confusing the isolation of penicillin with much later work on the chemical structure of penicillin to which, with an account of semi-synthetic penicillins, some 20 pages are devoted.

The first part of this book adds little to the description and interpretation of events in the UK save that it puts in better perspective the fundamental importance of N.G. Heatley's contribution to both the assay of penicillin and its extraction from crude culture medium. Its real strength lies in the second part, which deals with the development of penicillin in the United States after the visit by Florey and Heatley in the summer of 1941 to enlist the support of the American pharmaceutical industry. We are rightly reminded that even before that visit there had been some sporadic interest there in the phenomenon of antibiosis in general — Merck, for example, had supported S.A. Waksman at Rutgers University since 1939 — and penicillin in particular.

Dr Hobby herself, with M.H. Dawson and Karl Meyer at the College of Physicians and Surgeons in New York, experimented with crude penicillin very shortly after the publication of the first paper by the Oxford group in the *Lancet* of 24 August 1940. In 1944 she joined the pharmaceutical firm Pfizer as a microbiologist and is thus well qualified to recount the history of industrial production of penicillin in the USA. She also deals briefly with production in Canada, Holland, France, Germany and Japan. She brings out very clearly the dilemma of the American pharmaceutical industry during those critical years: heavy investment in fermentation plant might be rendered worthless almost overnight if the chemists came up with a viable synthesis — which in the event they did not.

One controversial issue is evaded, namely that process patents taken out by the US industry led to allegations in the UK Parliament and elsewhere that penicillin had been 'stolen' from Britain. Although these allegations were effectively disproved in a detailed inquiry initiated by Vannevar Bush in 1952, echoes of them are still heard. The overwhelming contribution of the USA to industrial production has encouraged popular belief there that penicillin was for all practical purposes an American discovery. Dr Hobby is at pains to dispel the illusion: "... penicillin was a British discovery — it was discovered in England, first studied in England, first used clinically in England — and it probably was one of Britain's major wartime contributions to society". □

Dr Trevor I. Williams, 20 Blenheim Drive, Oxford OX2 8DG, is editor of Endeavour. He worked in the Sir William Dunn School of Pathology, Oxford, 1942–5 and his biography 'Florey: Penicillin and After' was published by Oxford University Press in 1984.

Qwiffs and quantum leaps

Brian D. Josephson

Mind and the New Physics*. By Fred Alan Wolf. Heinemann: 1985. Pp. 342. £14.95.

IN THIS book the author expounds his ideas relating to a postulated connection between quantum mechanics and mind, a connection which is now beginning to be recognized as being possibly important by a number of scientists. Wolf starts from the conventional point of view that the quantum-mechanical wave function (or "qwiff" in his terminology) represents the potential for anything physical to become manifest, and regards the process of making decisions as one of interaction with the (potential) future. He postulates that we have control over the future by deciding what to observe, and thus determining which way the wave function should collapse. We capture possibilities by observing them and collapsing the wave function appropriately in the same way that a fisherman captures fish in his net. The author additionally discusses in a rather metaphorical way some information-processing aspects of quantum theory, as well as a large number of other, on the whole rather more questionable, ideas.

While the putative mechanism for being able to control the future has a certain degree of plausibility, conventional quantum theory would argue that the chance of *not* finding a particular possibility of interest should be taken into account as well as the one of actually finding it, these two possible outcomes being associated with wave function collapses in different directions. This would seem to lead to theoretical predictions distinctly different from those proposed by Wolf. His process for modifying the outcome would work only if a differential collapse mechanism were in operation, and would make the theory very close to E.H. Walker's theory of the control of physical reality by the will which was published in Vol. 3 of *Psychoenergetic Systems* (1979).

Unfortunately, the kind of attention to detail and use of logical methods of argument that might have made the author aware of matters such as the discrepancy between the mechanism he proposes and conventional theory is not a feature of this book. Its basic structure is one of informal expositions of standard theory, followed up by intuitions about their meaning in the author's scheme of things. Despite my sympathy with what Wolf is trying to do, it seems to me that the connections proposed, while occasionally being significant, have been arrived at mainly by a process of coupling together any two en-

ties that have some nebulous resemblance to each other (a typical example is the connection between Bose-Einstein statistics and love, discussed on pp. 152-154).

One can have little more confidence in the mathematical parts of the book, the illustrations for which have been produced using a computer apparently lacking graphics facilities, which hardly makes them easier to follow. Starting with an elementary howler in the example given to illustrate the addition of two wave functions on p. 191, which might be excused as a momentary lapse of attention, the author goes on to describe the equation $\langle 1/I \rangle = 1$ as representing the law "The Mind is One". He then proceeds further to equations supposedly representing the characteristics of Enterprise, God and Satan, at which point the book starts to bear a distinct resemblance to some of the more dubious offerings from the public that arrive occasionally in one's mail box. It could be that there is some sense behind these strings of equations, but unfortunately Wolf does not consider it important to go into the details so that the critical reader can judge for himself.

The side effect of books such as this may be to cause other books and journal articles, which treat this subject in a more serious manner, to miss the attention they deserve. There come to mind Rupert Sheldrake's *A New Science of Life*, which has been criticized mainly on account of its unorthodoxy and not because of any errors or inconsistencies in its arguments; Fritjof Capra's *Tao of Physics*; D. Bohm and B.J. Hiley's article (*Fdn Phys. USA* **14**, 255-274; 1984) which indicated that mind-like characteristics of nature may be inherent in the equations of quantum physics; and the two papers of H.P. Stapp (*Fdn Phys. USA* **12**, 363-399; 1982 and **15**, 35-47; 1985) on the connection between mind, matter and quantum mechanics. On the assumption that important new ideas may in fact be buried in Wolf's book, one must hope that a subsequent, more rigorous account will be written, directed at the scientist rather than at the non-scientist who seems to be the target of this one.

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Taking an aim

Gregory Gregoriadis

Red Blood Cells as Carriers for Drugs. Edited by J.R. DeLoach and U. Sprandel. Karger: 1985. Pp. 162. DM 125, \$44.25.

THE development of carriers to obtain optimal release of drugs where they are needed, and protection from hostile environments or premature loss, is a subject of growing interest and a hopeful bet in biotechnology ventures. Currently popular carriers include antibodies, polymers, liposomes and other colloids. With only a minority of workers favouring erythrocytes, what then is so special about them to warrant a meeting and a book? Several things according to the editors and the contributors. Most medical researchers are familiar with erythrocytes, a known quantity to be trusted, by clinicians especially (the danger of unwanted contaminants in other people's blood, is avoided by using the patient's own erythrocytes).

Erythrocytes can, in theory at least, optimize drug action in more than one way. For instance, they end up sooner or later in the reticuloendothelial system which is associated with a large number of microbial and other diseases, and could therefore carry drugs there. This aspect is discussed in chapters on the use of cells loaded with metal chelators which facilitate removal of stored iron, or with hydrolytic enzymes which can break down stored substrates in the tissues of patients with storage diseases. Further, (human) erythrocytes have a life span of over 100 days, an important feature for slow re-

lease of drugs into the circulation

The book describes experiences with the release of antimicrobial and anticancer drugs from circulating erythrocytes. Inactivation of toxic metabolites entering erythrocytes by enzymes which have been introduced into the cells is discussed in chapters on asparaginase, rhodanese (co-entrapped with sodium thiosulphate for cyanide detoxification), or urate oxidase-containing erythrocytes. Other applications include erythrocyte-mediated introduction of DNA and antibodies into eukaryotic cells and the enrichment of erythrocytes with allosteric effectors of haemoglobin or with 6-phosphate dehydrogenase.

Is this all too good to be true? Unfortunately yes. The editors and some of the authors recognize the formidable problems with the proposed uses, especially in terms of wide acceptability. For instance, there are already a number of simple and safe man-made carriers which can deliver drugs to the reticuloendothelial system efficiently. Further, release of drugs from circulating cells at a constant rate will probably be impossible to achieve. Perhaps the greatest advantage of erythrocytes over other systems is their long circulation time and their use in detoxification looks realistic enough. The book, discussing most aspects of the erythrocyte carrier and related technology in 19 short chapters, is the first one devoted entirely to this system and should prove useful to those interested in drug delivery.

Gregory Gregoriadis is Head, Medical Research Council Group, Academic Department of Medicine, Royal Free Hospital School of Medicine, London NW3 2QG, UK.

* In the United States *Star Wave* published by Macmillan, New York: Hbk \$19.95, pbk \$7.95.

Putting flesh to the phantoms

Graham Burton & D.D.M. Wayner

Free Radicals in Biology and Medicine. By Barry Halliwell and John M.C. Gutteridge. Clarendon: 1985. Pp.346. £30, \$45.

THE transitory nature of free radicals, conferred on them by an unpaired valence electron, has made direct proof of their involvement in a chemical reaction a challenging task. This is especially so within the complex web of chemistry associated with biological systems. Nevertheless, there is now strong, albeit indirect, evidence that free radicals are involved in at least some normal and abnormal cellular processes, including, for example, photosynthesis, inflammation, cancer and toxic reactions to some drugs and pesticides. Research interest in free radicals in biology and medicine has now grown to the extent that there are entire conferences and journals devoted to the subject.

Anyone wishing to gain an appreciation of the state of knowledge in this diverse, interdisciplinary field, together with a feel for the difficulties involved in pinpointing the *in vivo* involvement of radicals, will welcome the arrival of this very readable book. Furthermore, the authors have realized that further progress will require

a better understanding of the chemistry of free radicals. Accordingly, they have made accommodations in the text and included an appendix on bonding and atomic structure to help those readers who lack the necessary background. However, the chemically naive may find the number of chemical reactions and structures daunting (and will not be helped by errors in the printed structures of some chemical intermediates).

More importantly, the speculative nature of some of the reaction schemes is not always indicated. For example, two mechanisms are presented for the trapping of the superoxide ion by the antioxidant vitamin E (p.62 and p.173); not only are they contradictory, but both seem unlikely because they involve electron and proton transfers which have never been demonstrated experimentally and are improbable on thermodynamic and kinetic grounds. A more likely possibility is that the small amount of the more reactive hydroperoxyl radical that is in equilibrium with superoxide ion at physiological pH will rapidly abstract a hydrogen atom from vitamin E, as is found to occur with other peroxy radicals.

Reflecting the thrust of much of the research in this field, the authors devote considerable space to the potential roles played by the superoxide ion, hydrogen peroxide, the hydroxyl radical, iron and other transition metals, and the enzymes superoxide dismutase, catalase and glutathione peroxidase. They also describe the complex interplay of enzyme systems and the importance of maintaining sufficient concentrations of cofactors such as reduced glutathione and NADH. The reader, however, is left with the impression that the very reactive hydroxyl radical is the primary agent of destruction; this may be true, but it is also important to realize that the chain-carrying peroxy radicals (which will almost certainly form as a result of hydroxyl radical attack on neighbouring molecules) can lead to a much larger increase in the initial damage. In this case, other antioxidant systems (for example vitamin E, vitamin C) become important.

There is no direct referencing within the body of the text (except where individual researchers are named) but each chapter is supplemented with an alphabetical list of selected references (up to and including 1984) for further reading. This system has its advantages, but will frustrate those pursuing specific points in the text.

Although the book is aimed at biologists and clinicians, it is a good starting point for anyone wishing to get a clear introduction to current aspects of free radical research in biology and medicine. It should, therefore, be of wide appeal. □

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Adding up parts of the past

I Grattan-Guinness

A History of Algebra from al-Khwārizmī to Emmy Noether. By B.L. van der Waerden. Springer 1985; Pp.271. DM 98, \$34.50.

ALGEBRA has long been a major component of mathematics, and so has attracted a good deal of interest among historians. This book occupies a special place, for its author has been one of the most distinguished algebraists of our century. After a chapter on medieval Muslim contributions, he takes the story through Renaissance Italy to Descartes, and then on to the algebraic structures that became a 19th-century concern: the work of Gauss and Galois, then the development of group theory, and the various algebraic systems which culminated in a sort of synthesis with van der Waerden's teacher, Noether.

As algebra has already been studied by many other historians, several parts of this book cover familiar ground: its novelties are drawn from recent research by others on various historical figures, which is used heavily and with full acknowledgement. While no major recasting of the history is thereby caused, the new details are very welcome. However, when one considers the interaction between algebra and other branches of mathematics, gaps in the story do emerge, which neither this nor other histories properly fills. I shall mention two.

One of the chief links for algebra has been with calculus, and its uses in applied mathematics. Indeed, a major issue there at times from the mid 18th century onwards was the possibility of securing some of these topics entirely within certain algebraic theories. While these attempts were successful, some important consequences followed for algebra: for example, they led to the early stages of functional equations and differential operators, the first theories in algebra explicitly divorced from interpretations in terms of length and number.

Partly as a result of these attempts, the 19th century saw the introduction of a variety of algebras in which laws other than the usual ones, suggested by arithmetic, were satisfied. For some reason the English became especially attracted to such studies, which gained collective prominence in their time, both in England and to some extent abroad. It is therefore rather astonishing that van der Waerden deals only with Hamilton, and that his book is completely silent on Babbage, Boole, Gregory, Herschel, De Morgan and Sylvester. The history of the history of algebra still has much life left in it. □

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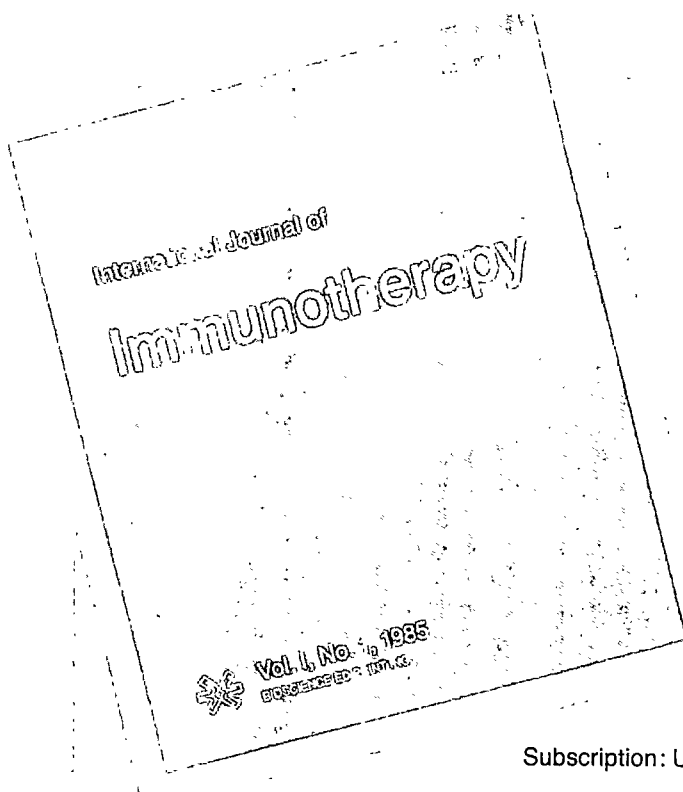
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Vaccination and herd immunity to infectious diseases

Roy M. Anderson & Robert M. May*

Department of Pure and Applied Biology, Imperial College, London University, London SW7 2BB, UK

* Biology Department, Princeton University, Princeton, New Jersey 08540, USA

An understanding of the relationship between the transmission dynamics of infectious agents and herd immunity provides a template for the design of effective control programmes based on mass immunization. Mathematical models of the spread and persistence of infection provide important insights into the problem of how best to protect the community against disease.

MUCH attention is now focused on the development and community-wide use of vaccines for the control of infectious diseases. This interest is a consequence of many factors; these include the worldwide eradication of smallpox by vaccination in the late 1970s¹, the current success of child immunization programmes for the control of measles, mumps, pertussis and rubella in the United States^{2,3}, the failure of frequent appeals by public health authorities in the United Kingdom to raise vaccination rates that are at present low by developed-country standards⁴⁻⁷, the Expanded Programme on Immunization (EPI) of the World Health Organisation (WHO) for the control of a variety of serious childhood viral and bacterial diseases in the developing world⁸⁻¹², and the rapid advances in molecular biology and biotechnology which promise new vaccines for the future¹³⁻¹⁶. The main thrust of research today is at the molecular level, where it is stimulated by the urgent need to develop vaccines for the major killing diseases of the Third World such as malaria¹⁷⁻¹⁹, and to combat new infections such as the current epidemic of AIDS (acquired immune deficiency syndrome), caused by the recently isolated human T-lymphotropic virus (HTLV-III)^{20,21}.

The development of a safe, effective and cheap vaccine, however, is only a first step (albeit an essential one) towards community-wide control. Epidemiological, economic and motivational issues are at least as important as technological ones. A vaccine for measles (a very cost-effective immunization¹²) has been available since the late 1960s, yet the infection remains one of the world's major causes of child mortality^{8,22}. Concomitant with research on vaccine development, there is a need for an improved understanding of how best to use vaccines to protect the community as well as the individual. The persistence of infectious disease within a population requires the density of susceptible individuals to exceed a critical value such that, on average, each primary case of infection generates at least one secondary case. It is therefore not necessary to vaccinate everyone within a community to eliminate infection; the level of herd immunity must simply be sufficient to reduce the susceptible fraction below the critical point. The central epidemiological questions are thus: what proportion of the population should be vaccinated to achieve elimination (in a local programme), eradication (in a global programme) or a defined level of control? How is this affected by demographic factors (for example, birth rate)? What is the best age at which to immunize? How does mass immunization affect the age distribution of susceptible individuals, particularly in those classes most at risk from serious disease, and how important is genetic and spatial heterogeneity in susceptibility to infection (or response to immunization) to the creation of effective herd immunity²³⁻²⁶?

To answer these questions, we need to understand the interaction between the transmission dynamics of the disease agent and the level of naturally acquired (or artificially created) immunity to infection. This relationship is complex and depends on such factors as the precise course of infection within an individual, the demography of the host population, the duration

of acquired immunity and maternally derived protection, age-related changes in the degree and intimacy of contacts among people, and the prevailing levels of genetic, spatial and behavioural heterogeneity in susceptibility/resistance to infection. Intuition built on many years of practical epidemiological experience often fails to predict the outcome of a particular vaccination programme. Mathematical models which clearly and accurately define the details of the association between disease agent and host (at the individual and population levels) can help to overcome such difficulties. This article reviews recent theoretical and empirical work on the transmission dynamics of infectious disease. Emphasis is placed on the relevance of such research to the design of disease control programmes based on vaccination.

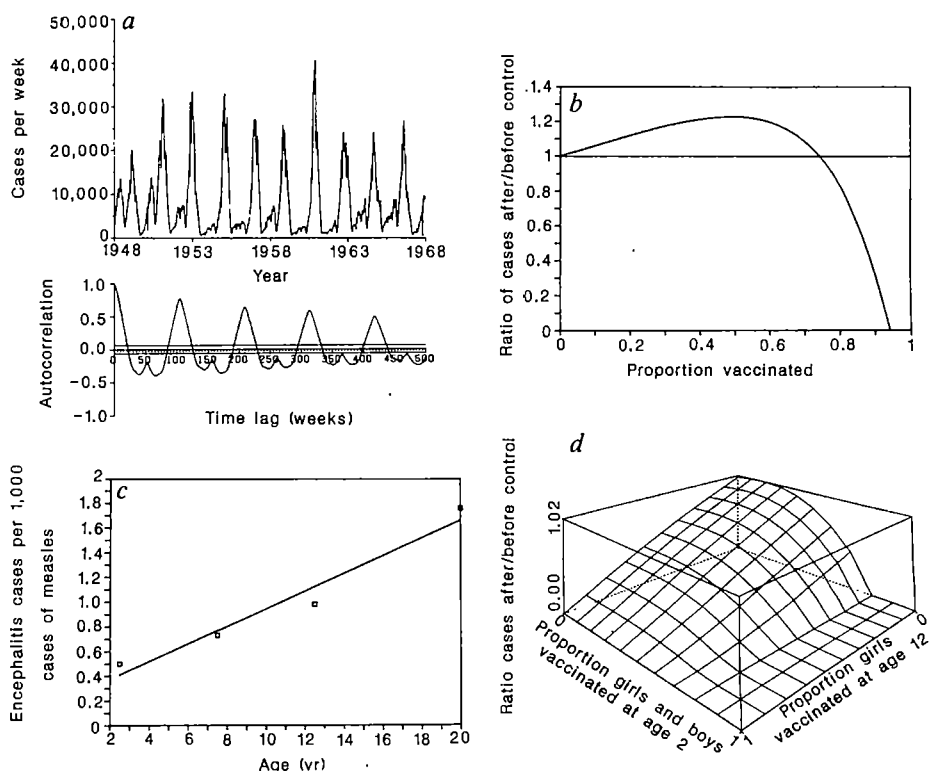
General theory

Models for the transmission dynamics of directly transmitted viral and bacterial infections are typically compartmental in structure²³⁻³⁵. They represent changes, with respect to age, a , and time, t , in the populations of infants protected by maternally derived antibodies ($M(a, t)$), susceptible individuals ($X(a, t)$), infected individuals who are not yet infectious ($H(a, t)$), infectious people ($Y(a, t)$), and people who have recovered and are immune from subsequent infection ($Z(a, t)$). The total population is $N(a, t) = M(a, t) + X(a, t) + H(a, t) + Y(a, t) + Z(a, t)$. The deterministic equations describing the flows from one class to another are of the general form^{24,25,27}

$$\begin{aligned} \frac{\partial M(a, t)}{\partial t} + \frac{\partial M(a, t)}{\partial a} &= -[\mu(a) + d]M(a, t) \\ \frac{\partial X(a, t)}{\partial t} + \frac{\partial X(a, t)}{\partial a} &= dM(a, t) \\ &\quad - [v(a, t) + \lambda(a, t) + \mu(a)]X(a, t) \\ \frac{\partial H(a, t)}{\partial t} + \frac{\partial H(a, t)}{\partial a} &= \lambda(a, t)X(a, t) \\ &\quad - [\mu(a) + \sigma]H(a, t) \\ \frac{\partial Y(a, t)}{\partial t} + \frac{\partial Y(a, t)}{\partial a} &= \sigma H(a, t) - [\mu(a) + \gamma]Y(a, t) \\ \frac{\partial Z(a, t)}{\partial t} + \frac{\partial Z(a, t)}{\partial a} &= \gamma Y(a, t) + v(a, t)X(a, t) \\ &\quad - \mu(a)Z(a, t) \\ \frac{\partial N(a, t)}{\partial t} + \frac{\partial N(a, t)}{\partial a} &= -\mu(a)N(a, t) \end{aligned} \quad (1)$$

Here $\mu(a)$ denotes age-related human mortality, $1/d$ is the average duration of protection provided by maternal antibodies, $1/\sigma$ and $1/\gamma$ are the average incubation and infectious periods respectively, and $v(a, t)$ records the age- and time-specific vaccination schedule. Mortality explicitly associated with the

Fig. 1 *a*, Time series analysis of the case reports for measles in England and Wales over the pre-vaccination period 1948–68. The top graph denotes the recorded cases and the bottom graph the serial correlogram⁵¹. Note the significant yearly (seasonal) and 2-yearly peaks in incidence (the horizontal axis in the bottom graph denotes weeks). *b*, Model predictions of the equilibrium (the state to which the system settles in the long term) ratio of the number of cases of mumps in the age range 14–50 yr (the age range in which males are at risk to serious disease resulting from infection) under the impact of a vaccination programme in which *p* of each yearly cohort are vaccinated at age 2 yr, divided by the number of cases in the same age range before vaccination. If the ratio is above unity, the control programme has a detrimental effect and vice versa. The average age at infection before control was assumed to be 6.5 yr (appropriate for the United Kingdom) and the calculations were based on a model defined in ref. 24. The straight line denotes the ratio of unity. *c*, Age-specific incidence of reported measles encephalitis cases (defined per 1,000 cases of measles) in the United States in 1973–79¹⁶. *d*, Similar to *b* but representing the ratio of cases after vaccination divided by cases before vaccination for rubella in the United Kingdom in the age range 16–40 yr. The graph denotes model predictions (equilibrium values) for a two-stage vaccination policy involving vaccination of only girls at age 12 yr (the UK policy) and vaccination of boys and girls at age 2 yr (the US policy)^{25,67}. Note that the addition of a second-stage US policy over the current UK policy is only of substantial benefit if moderate to high levels of vaccination coverage are achieved at 2 yr of age. The average age at infection before control was assumed to be 9–10 yr of age^{25,67}.



infection has been ignored. The term $\lambda(a, t)$ denotes the 'force' or rate of infection, which under the 'mass action' assumption adopts the form^{24–30} $\lambda(a, t) = \int_0^\infty \beta(a, a') Y(a', t) da'$. Here $\beta(a, a')$ represents the transmission coefficient for infected individuals in age class a' who come into contact with susceptible individuals in age class a (refs 25, 30). The boundary conditions of equations (1) depend on the demography of the community. The structure of the model may be modified to mimic the transmission of a wide variety of infections^{31–34}, including those with complex life cycles (for example, malaria or yellow fever^{35–37}) and those involving carriers (such as tuberculosis and hepatitis B³⁸) or macroparasites (such as helminths) where note must be taken of the distribution of parasite numbers within the human community^{39,40}.

Insights into applied problems of disease control or into general ecological and epidemiological questions can be obtained through analytical or numerical studies of equations (1).

Cycles in incidence

For most common viral and bacterial infections of childhood, the model predicts large-amplitude, slowly damping oscillations in disease incidence^{23–30}. Several factors can tend to perpetuate these oscillations indefinitely; these are stochastic effects^{41–44}, age-dependent transmission rates²⁵, incubation periods of a discrete nature^{45,46} and seasonality in contact^{27,47–50}. The average period of oscillations, T , is approximately given as²³: $T \approx 2\pi(AK)^{1/2}$, where A is the average age at infection and K is the generation time of the infection ($K = 1/\sigma + 1/\gamma$).

The observed periods of such cycles (which agree well with prediction) can be determined accurately by time-series techniques (Table 1a, Fig. 1a⁵¹); the longer-term cycles are usually compounded with short-term seasonal effects.

Vaccination and herd immunity

The basic reproductive rate or transmission potential of an infection, R_0 , is the average number of secondary cases produced by one primary case in a wholly susceptible population^{33,34}. Clearly, an infection cannot be maintained unless R_0 exceeds

unity. Within communities in which the infection is endemic, R_0 is inversely correlated with A , the average age at infection^{23–37} ($R_0 \approx B/(A - D)$). This relation holds both for developed countries (growth rates essentially zero) and for developing countries (with their growing populations)⁵². Here B is the reciprocal of the finite birth rate (usually expressed as births (yr^{-1}) per 1,000 of population), and D is the duration of maternally derived immunity. The value of R_0 within a community is critically dependent on social and behavioural factors (the contact component of β which influences λ and hence A), on the biology of the disease agent and host (the infectiousness and susceptibility components of β) and on the demography of the community (value of B)²⁵. The marked difference between developed and developing countries in the average ages at which children typically acquire infections such as measles in urban communities is a consequence of variations in social, behavioural and demographic factors (Table 1b).

To eradicate an infection by mass immunization it is necessary to reduce the value of R_0 below unity^{23–25,53}. This can be achieved by vaccinating a proportion, p , of each cohort of the population at age V (where $V > D$; vaccination during the period of maternally derived protection usually fails to protect the child adequately from subsequent infection^{54,55}) provided^{23,25} $p > [1 - A/B]/[1 - V/B]$. The infection can never be eradicated if $V > A$ (that is, $p > 1$). The value of p is minimized by vaccinating at as young an age as possible, given the constraint of $V > D$. These conclusions are derived under the assumption that the rate of transmission within the community is fairly homogeneous. In practice, this assumption is often violated^{25,30}, but simple theory provides a useful guide, usually setting an upper limit to the levels of vaccination required to eliminate many common childhood infections²⁵. It forms the basis of estimates suggesting that 92–96% of children must be vaccinated to eliminate measles and pertussis, 84–88% to eliminate rubella and 88–92% to eliminate mumps in Western Europe and the United States; these estimates assume that vaccination takes place just after the wane of maternal antibody protection^{23–25}. Recent experience in the United States suggests that high levels of cohort immunization are required (as indicated by theory)

Table 1 Infections reported throughout the world

<i>a</i>			<i>b</i>						
Infectious disease	Inter-epidemic period (yr)	Geographical location and time period	Infectious disease	Average age <i>A</i> (yr)	Geographical location and time period				
Measles	2	England & Wales, 1948–68	Measles	5–6	USA, 1955–58				
	2	Aberdeen, Scotland, 1883–02		4–5	England & Wales, 1948–68				
	2	Baltimore, USA, 1900–27		2–3	Morocco, 1962				
	2	Paris, France, 1880–10		2–3	Ghana, 1960–68				
	1	Yaounde, Cameroun, 1968–75		2–3	Pondicherry, India, 1978				
	1	Ilesha, Nigeria, 1958–61		1–2	Senegal, 1964				
Rubella	3–5	Manchester, UK, 1916–83	Rubella	1–2	Bankok, Thailand, 1967				
	3–5	Glasgow, Scotland, 1929–64		9–10	Sweden, 1965				
Parvovirus (HPV)	3–5	England & Wales, 1960–80		9–10	USA, 1966–68				
Mumps	3	England & Wales, 1948–82		9–10	Manchester, UK, 1970–82				
	2–4	Baltimore, USA, 1928–73		6–7	Poland, 1970–77				
Poliomyelitis	3–5	England & Wales, 1948–65		2–3	Gambia, 1976				
Echovirus (type II)	5	England & Wales, 1965–82	6–8	USA, 1912–28					
Smallpox	5	India, 1868–48	12–17	USA, 1955					
Chickenpox	2–4	New York City, USA, 1928–72	Pertussis	4–5	England & Wales, 1948–68				
	2–4	Glasgow, Scotland, 1929–72	4–5	USA, 1920–60					
Coxsackie virus (type B2)	2–3	England & Wales, 1967–82	Mumps	6–7	England & Wales, 1975–77				
Scarlet fever	3–6	England & Wales, 1897–78		6–7	Netherlands, 1977–79				
Diphtheria	4–6	England & Wales, 1897–79	<i>c</i>						
Pertussis	3–4	England & Wales, 1948–85	Force of infection λ (yr ⁻¹)	Age groups (yr)					Proportion to be immunized for eradication (<i>p</i>)
<i>Mycoplasma pneumoniae</i>	4	England & Wales, 1970–82		0–5	5–10	10–15	15–20	20–75	
			Case 1	0.2	0.2	0.2	0.2	0.2	0.94
			Case 2	0.18	0.56	0.2	0.1	0.1	0.89

a, The inter-epidemic period, T , for various viral and bacterial infections^{23–25,51,99,115}. b, Average age at infection (yr), A , for different diseases in different localities^{23,24,34,99,116}. c, The impact of age-related changes in the force of infection, λ (yr⁻¹), on the critical proportion of the population p , to be vaccinated to eradicate measles in England and Wales^{23–26} (case 1, constant; case 2, age dependent).

to substantially reduce the incidences of common childhood infections.

The vaccination of cohorts must be continued for many decades before the full impact of a programme is manifest; long-term suppression or interruption of transmission requires continual cohort vaccination as long as the infection remains endemic in other communities or countries. The only infection to be eradicated worldwide is smallpox. This success (announced by WHO in 1977) generated some optimism concerning the feasibility of eradicating other major infections, such as measles and pertussis^{8,9,56–58}. Such optimism, however, may be misplaced, given the low communicability of smallpox (low R_0 and high average age at infection, A , before widespread immunization) in comparison with infections such as measles, the ease with which smallpox infection can be diagnosed, the practical simplicity of inoculation procedures and the stability of the smallpox vaccine under primitive storage conditions^{1,59,60}.

Successful immunization programmes may themselves create health problems, since vaccination almost invariably carries some risk (usually extremely small) to the individual. At the start of a mass-immunization programme, the probability of serious disease arising from vaccination is usually orders of magnitude smaller than the risk of serious disease arising from natural infection. As the point of eradication is approached, the relative magnitude of these two probabilities must inevitably be reversed. The optimum strategy for the individual (not to be vaccinated) therefore becomes at odds with the needs of society (to maintain herd immunity). This issue—which was central to the decline in uptake of whooping cough vaccine in the United Kingdom during the mid- to late-1970s—can be overcome by legislation to enforce vaccination (as in the United States), but its final resolution is only achieved by global eradication of the disease agent (so that routine vaccination can cease)⁶¹.

Mass immunization at levels below that required for elimination acts to reduce the number of cases of infection (and hence the force of infection, λ). However, both theory^{23–26} and observation^{62,63} suggest that it has little, if any, impact on the total number of susceptible individuals. If the infection is endemic, its effective reproductive rate is unity—each case produces, on average, one other case—and the fraction susceptible remains roughly constant ($\sim 1/R_0$), independently of whether suscepti-

bility is lost by vaccination or by natural infection. Reduction of transmission via vaccination, however, usually increases the inter-epidemic cycle period and the average age at infection^{23–26,51}. This latter manifestation can be worrying if the risk of serious disease resulting from infection increases with age (Fig. 1c). Whether this issue is important depends on the quantitative details of such factors as how risk changes with age, the average age at which the vaccine is administered, and how the force of infection changes with age before widespread immunization^{25,26} (Fig. 3a). Rubella and mumps are of particular concern because of the risk of congenital rubella syndrome (CRS) in infants born to mothers who contracted rubella in their first trimester of pregnancy^{64,65} and the occurrence of orchitis and the associated risk of sterility in post-pubertal males following mumps infection⁶⁶ (Fig. 1b, c). The problems surrounding mass vaccination against rubella (to control CRS) have resulted in the adoption of different vaccination programmes in different countries. In the United Kingdom, girls only are vaccinated at an average age of around 12 yr, so as to allow rubella virus to circulate and create naturally acquired immunity in the early years. By contrast, in the United States both boys and girls are vaccinated at around 2 yr of age, with the aim of eliminating rubella. Theory suggests that the US policy is best if very high levels of vaccination (80–85% of each yearly cohort) can be achieved at a young age, while the UK policy is better if this cannot be guaranteed^{24,67}. A mixed US and UK policy is predicted to be of additional benefit over the UK single-stage policy if moderate to high levels of vaccine uptake among boys and girls can be achieved at a young age ($>60\%$)⁶⁷ (Fig. 1d).

Epidemiological data

The average age at infection, A , is central to most estimates of the intrinsic transmission potential of an infection, to the calculation of the desired level of vaccination coverage for elimination, and to theoretical assessment of how the risk of serious disease caused by infection may change with age under the impact of mass vaccination. For infections which are thought to induce life-long immunity against disease (for example, measles, mumps, rubella and pertussis), the value of A can be estimated (see Table 1b) either from age-stratified case notification records or, more accurately, from serological profiles (Fig. 2).

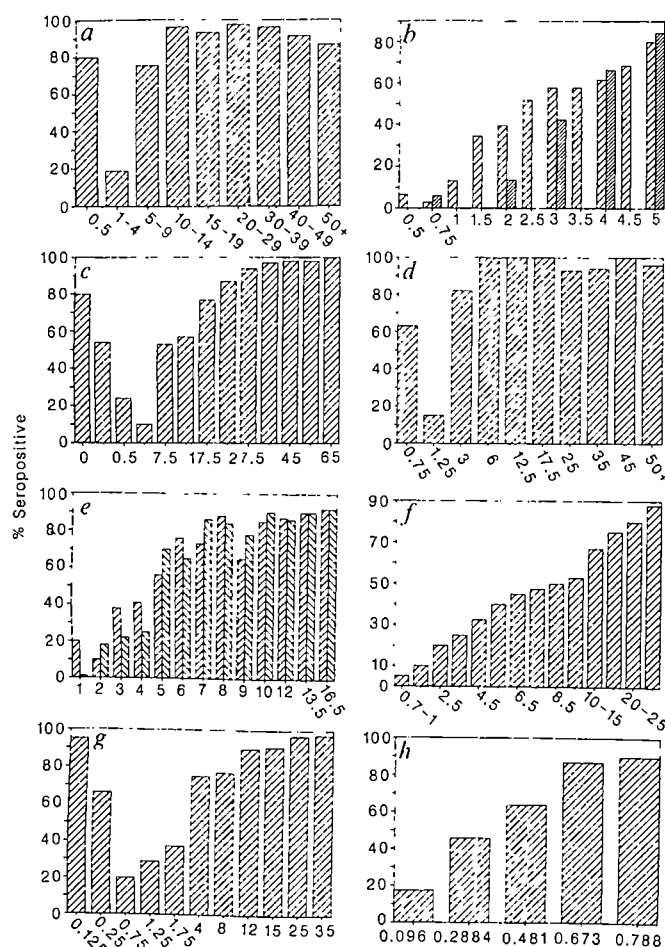


Fig. 2 Age-stratified (horizontal) serological profiles of the percentages of people in different age groups with antibodies to various infectious disease agents in developed and developing countries. *a*, Measles in the United States in 1955-58 (New Haven, Connecticut)¹¹⁷. *b*, Measles in India¹¹⁸ (left-hand histograms) and Nigeria¹¹⁹ (right-hand histograms). Note the rapidity with which the proportion seropositive rises with age when compared with *a*. *c*, Rubella in England and Wales²⁴ in the 1960s and 1970s. *d*, Rubella in the Gambia in the late 1970s¹²⁰. Again note the rapidity of the rise in seropositivity with age when compared with *c*. *e*, Mumps in England and Wales (left-hand histograms) and the Netherlands (right-hand histograms)^{121,122}. *f*, Polio in the United States in 1955-58³⁴. *g*, Hepatitis B (HBV) in Senegal¹²³. The vertical axis denotes the percentage with HBV markers. *h*, Malaria (*Plasmodium falciparum*) in Nigeria¹¹⁵. The vertical axis denotes the cumulative percentage of children who have experienced infection. Note that in *a-h* the scales of the x-axes are not always identical and in certain cases record unequal age class divisions (a nonlinear scale).

The interpretation of the data, however, is beset with many problems, such as age-specific biases in notification records^{68,69}, and a reduced ability to detect antibodies in adults who experienced infection in early childhood⁷⁰.

Heterogeneity in transmission

Conventional epidemiological theory is founded on the 'mass action' principle of transmission (see equations (1)), in which infection is assumed to spread via contacts within a homogeneously mixing community⁷¹. Recent research has begun to take account of the complications induced by heterogeneity in transmission arising from age-related, genetic, spatial or behavioural factors^{25,30,72-77}.

Age-dependent factors. Analyses of case notification records and serological profiles suggest that, for many common infections (measles, rubella and pertussis), the per capita rate of infection ($\lambda(a, t)$) depends on the ages of susceptible individuals, changing from a low level in the 0-5-yr age classes, via a high level in the 5-15-yr age classes, back to a low level in the adult age classes (Fig. 3a)²⁵. This is of interest both because it reflects behavioural attributes of human communities and because of

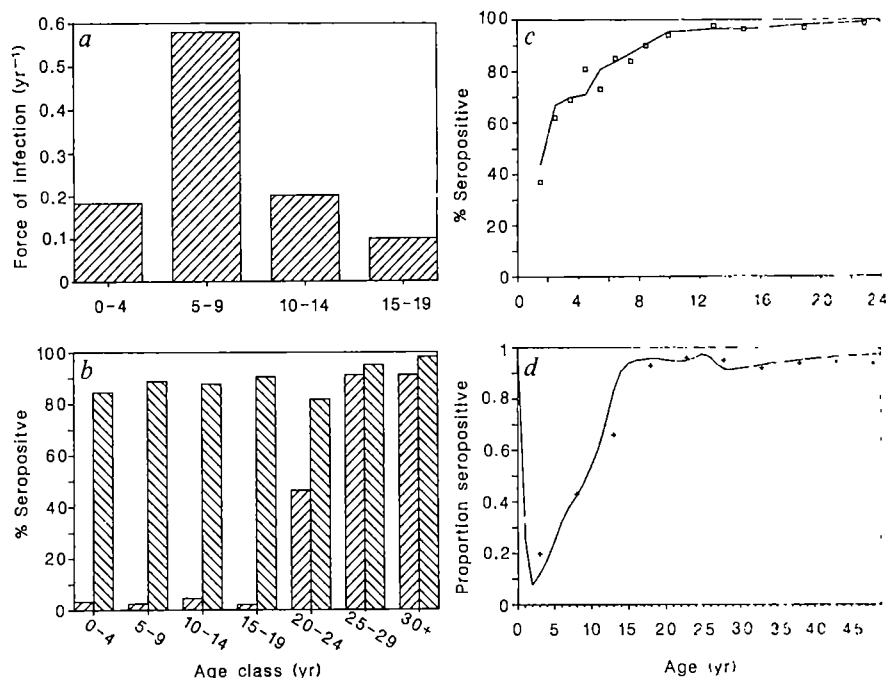
its impact on the level of vaccine-induced herd immunity required to eliminate an infection^{25,67,75}. The high values of λ observed in the 5-15-yr-old classes (Fig. 3a) are thought to arise as a consequence of frequent and intimate contacts within school environments.

Analyses of the significance of age-related changes in contact with infection are crude at present, due to the absence of detailed information on 'who acquires infection from whom'²⁵. Models which incorporate high degrees of contact within school-age classes (and low contact in the very young and in adult age groups), however, yield predictions of temporal changes in disease incidence and of age-related changes in serology in vaccinated versus unvaccinated communities which closely parallel observed trends²⁵ (Figs 3c, d, 4a-c). Recent numerical studies suggest that predictions based on the assumption of homogenous mixing tend to overestimate the critical level of vaccination coverage for elimination by roughly 5% for infections such as measles and pertussis in the United Kingdom and United States (Table 1c)^{25,30}. For these infections, analyses based on apparent age-related changes in contact with infection (Fig. 3a) yield estimates of approximately 90% coverage at 1-2 yr of age²⁵. These predictions, however, must be accepted with caution at present, because the observed low rates of infection in late teenage and adult age groups may be artefacts arising from biases in case reporting, from the insensitivity of serodiagnostic tests, or from genetic variability in susceptibility to infection⁷⁸. These caveats are underlined by the fact that no such marked age-related changes in the per capita rate of infection are found in epidemics within virgin populations on islands (largely susceptible to infection before the epidemic⁷⁹⁻⁸¹) (Fig. 3b). At present it seems prudent to accept the higher vaccine coverage levels estimated by homogeneous mixing models²⁵.

Genetic factors. Genetic factors are important in determining host susceptibility and resistance to infectious agents⁷⁸. Genetically based variability in the level and duration of antibody production following infection would seriously complicate the interpretation of age-serological profiles⁸². Associations between HLA type and antibody titres following vaccination against rubella and measles provide evidence for the existence of such complications^{83,84}. Little is understood, at present, about the importance of such factors in the design of vaccination programmes. In the future, however, community-based diagnostic programmes aimed at the identification of immunodeficiency could make it possible to target vaccination, for certain less-common but serious viral and bacterial diseases, to those children genetically predisposed to infection and severe morbidity⁸⁵.

Spatial factors. Inhomogeneity in the spatial distribution of hosts, with some people living in dense aggregate and others living in isolated or small groups, can lead to heterogeneity in transmission rates. This, in turn, can result in the transmission potential of an infection (R_0) being greater on average than suggested by estimation procedures which assume spatial homogeneity^{86,87}. Under these circumstances, the optimal solution appears to involve 'targeting' vaccination coverage in relation to group size, with dense groups receiving the highest rates of vaccination⁷⁴. The optimal programme is defined as that minimizing the total, community-wide number of immunizations needed for elimination or a defined level of control. This strategy reduces the overall proportion that must be vaccinated to achieve elimination, compared with that estimated on the assumption of spatial homogeneity. This conclusion has practical significance for the control of infections such as measles and pertussis in some developing countries, where rural-urban differences in population density tend to be much more marked than in developed countries. It is probable that in many regions of Africa and Asia, diseases such as measles cannot persist endemically in rural areas without frequent movement of people between rural and urban regions ($R_0 < 1$ in rural areas). Under these circumstances, disease control might be achieved in both types of region by high levels of mass immunization in the urban centres alone.

Fig. 3 *a*, Age-specific change in the rate, or force, of measles infection (λ) in England and Wales before mass vaccination²⁵. *b*, Percentages seropositive for rubella antibodies in various age classes on St Paul's Island, Alaska, before (left-hand histograms) and after (right-hand histograms) an epidemic of rubella in 1963⁷⁹⁻⁸¹. The population (~400 people) had not experienced rubella infection (before 1963) for 22 yr. Note that in the age range 0-19 yr, the rate of infection of susceptible individuals was similar in each age class. *c*, Comparison of model predictions (equations (1)) of the age-serological profile for measles antibodies in England and Wales with observed patterns. The observed data (squares) were collected in the period 1979-82. Model predictions were based on an average age of infection of 4.5 yr before control and the age-specific vaccination rates in the United Kingdom over the period 1968-82²⁵. *d*, Comparison of model predictions of the age-serological profile for rubella antibodies in females in England and Wales with the observed pattern (crosses) in 1984⁶⁷. Predictions were based on an average age of infection of 10 yr before control and the age-specific vaccination rates in the United Kingdom (vaccination of teenage girls between the ages of 10 and 15 yr) over the period 1970-84.



Behavioural factors. These are of great importance as determinants of disease transmission and provide a source of significant heterogeneity. The sexually transmitted infections provide a good example because the probability distribution of sexual promiscuity (defined as the number of different sexual partners per unit of time) is highly skewed. It seems very likely that the highly sexually active individuals ($R_0 \gg 1$) in the tail of the distribution are responsible for maintaining transmission within the community as a whole⁸⁸⁻⁹⁰. Drug treatment and surveillance focused on these 'superspreaders' is therefore highly beneficial, given that R_0 may be below unity for the remaining population. If vaccines are developed for HTLV-III, selective immunization of those most at risk⁹¹⁻⁹³ (haemophiliacs, intravenous drug abusers, persons of Central African or Haitian origin and homosexual males) and those predisposed to serious clinical infection, may be a practical option for community-wide control.

Behavioural factors are also important in helping to cause seasonal fluctuations in the incidence of many common viral and bacterial infections^{49,75,94}. The timings of school terms and vacation periods has a marked influence on temporal patterns of measles and pertussis cases in the United Kingdom and West Germany, for example.

Developing countries

Many infections that in developed countries are primarily a source of morbidity are major cause of mortality in the developing world (mainly as a consequence of malnutrition, although genetic components may also be involved)^{9,22,95}. Measles, for example, is estimated to cause more than one million child deaths annually^{9,22}. Although there is much current interest in worldwide immunization programmes for the control of such infections, the problems of creating sufficient levels of herd immunity for elimination should not be underestimated, given the logistic difficulty of vaccine storage and delivery in poor countries. Even if such practical issues are resolved by effective cold-chains, the development of heat-stable vaccines, and aerosol delivery techniques (which are more effective in the immunization of infants with maternal antibodies)⁹⁶⁻⁹⁸, the feasibility of blocking transmission by mass vaccination in areas where the average age of infection is often as low as 1-2 yr of age is questionable (Table 1b).

Consider a large urban centre in India or Africa, where the annual birth rate is around 40 per 1,000 population and the average age of measles infection is 2 yr (Fig. 2). Vaccination must take place after the maternally derived protection has

waned (6 months) and before the age of 2 yr to achieve elimination. Calculations suggest an R_0 value of around 17, which is similar to that for measles in England and Wales (with a birth rate of 12 per 1,000 and an average age of infection around 4.5-5 yr). Simple theory suggests that something like 96% of each yearly cohort of children would have to be effectively immunized by age 1 yr ($V=1$) to attain elimination. Coverage could be lower in rural areas but, as suggested in the section on spatial heterogeneity, an optimal control programme would have to focus on the urban centres. These estimates question the feasibility of measles elimination in many developing countries at this time and argue that, as a first priority, worldwide programmes should concentrate on controlling morbidity and mortality among those most at risk^{52,99}. Moderate to low levels of vaccination coverage, however, are beneficial; they raise the average age at infection and thereby lengthen the age 'window' in which vaccine can be administered⁹⁹, and reduce morbidity and mortality (young infants appear to be more at risk than older children).

New vaccines

The coming decade will see the development and use of a number of new vaccines, along with the expansion of community-based programmes of mass immunization using the currently available vaccines. The development of safe, effective and cheap vaccines is clearly the first priority, but, once these objectives are achieved, the problem of community-wide control requires careful attention. In particular, age-specific serological profiles of the community need to be obtained both before and during any programme of mass vaccination.

An example is provided by the recent proposal to initiate a mass-vaccination campaign for the control of hepatitis B virus (HBV) in Taiwan¹⁴ by a serum-derived vaccine (a genetically engineered vaccine is also available^{100,101}; Fig. 2). The epidemiology of HBV is made complicated by chronic infection in immunodeficient individuals who become carriers of the virus (with a high associated risk of inflammatory disease and malignancy)¹⁰², and who constitute an important reservoir of infection. The desirability of mass immunization is clear when viewed as a method for reducing the incidence of carriers (the risk of becoming a carrier appears to be much greater in children than in adults¹⁰³), but less clear given the observation that serious symptoms of disease are more likely to occur in adults than in children. Serological surveys in high-risk regions (South-East Asia and Africa) indicate that HBV typically has a low R_0 within the population as a whole (when compared with measles).

Moderate levels of vaccination coverage should therefore have a significant impact on disease incidence^{23,102,104} (Fig. 1d). The calculation of R_0 is made complicated by the presence of carriers ($R_0 = R_{01}f + R_{02}(1-f)$, where f is the fraction of carriers and $(1-f)$ the fraction of non-carriers) and a component of perinatal (=vertical) transmission⁴². The maintenance of transmission ($R_0 > 1$) is probably due to the carriers alone ($R_{01} \gg 1$, $R_{02} < 1$) given that they are often a significant fraction of the community (with f values ranging from 0.1% to 2% in the United States and most European countries, to as high as 50% in certain Pacific islands (determined in part by the ethnic composition of a community¹⁰²)).

Vaccines for viral infections such as cytomegalovirus, rhinovirus, adenovirus, rotavirus, respiratory syncytial virus and parainfluenza virus may provide sufficient protection to the individual to be considered for community-wide use in the future, provided their public health significance is of sufficient importance relative to other priorities in health care. The existence of many distinct antigenic types within certain groups of these viruses (for example, adenovirus and rhinovirus), however, present problems for vaccine development.

Recent work has raised the hope that vaccines may be developed for the major parasitic infections such as malaria (*Plasmodium falciparum*) and certain helminth species¹⁰⁵. The problems, however, are formidable because of the antigenic complexity of protozoa and helminths (in comparison with viruses and bacteria)¹⁰⁶, sequential changes in surface antigens during development within the host (for example, the filarial worms)¹⁰⁷, antigenic variation (whether due to the parasite's ability to express many variable antigen types, as in the case of the African trypanosomes^{108,109}, or as a consequence of population genetic (=strain) variability, as in the case of the malaria parasites¹¹⁰), and the failure of man naturally to develop fully protective immunity under conditions of repeated exposure to infection¹¹¹. Vaccines must be more effective in stimulating the host's immunological defences than the parasite itself if they are to overcome the evasion mechanisms that certain parasitic species have evolved¹¹². Combined with these problems is the suspicion that heterogeneity in immunological responsiveness is an important determinant of the observed aggregation in the distribution of parasite numbers per person (such that 15% of the hosts will often harbour 90% or more of the parasites)^{39,40,113,114}. Predisposition to heavy or repeated parasitic infection is often a consequence of behavioural or social factors but laboratory studies increasingly point to the importance of genetic and nutritional components¹¹². An effective vaccine must therefore be able to protect both immunocompetent and immunodeficient individuals, the latter being those most likely to suffer serious disease and to maintain parasite transmission.

The ideal malaria vaccine will probably contain antigens from different malaria species, strains and developmental stages (sporozoite, asexual and gamete) so as to reduce mortality and morbidity, and to block transmission^{18,19}. But once the technical problems in vaccine development have been overcome, the issues of community control must take priority. The creation of a level of herd immunity high enough to eliminate malaria appears impractical in endemic regions where the average age at first infection is typically around 3–6 months of age ($R_0 = 50$ –100, which implies 99% coverage of the community before the age of 3 months with a vaccine which gives lifelong protection) (Fig. 1e). Low to moderate levels of immunization, however, will greatly help to control mortality and morbidity, which is of greatest significance in the 0–5-yr-old age classes. Care must, however, be exercised to prevent an associated build-up of susceptible individuals in teenage and adult age classes; at present, such individuals have acquired a degree of immunity to infection from repeated exposure in childhood. Once the properties of a potential vaccine are more clearly defined, mathematical studies can help to quantify such risks. In the case of helminth infections, vaccines will probably only provide protection of short duration against re-infection, and

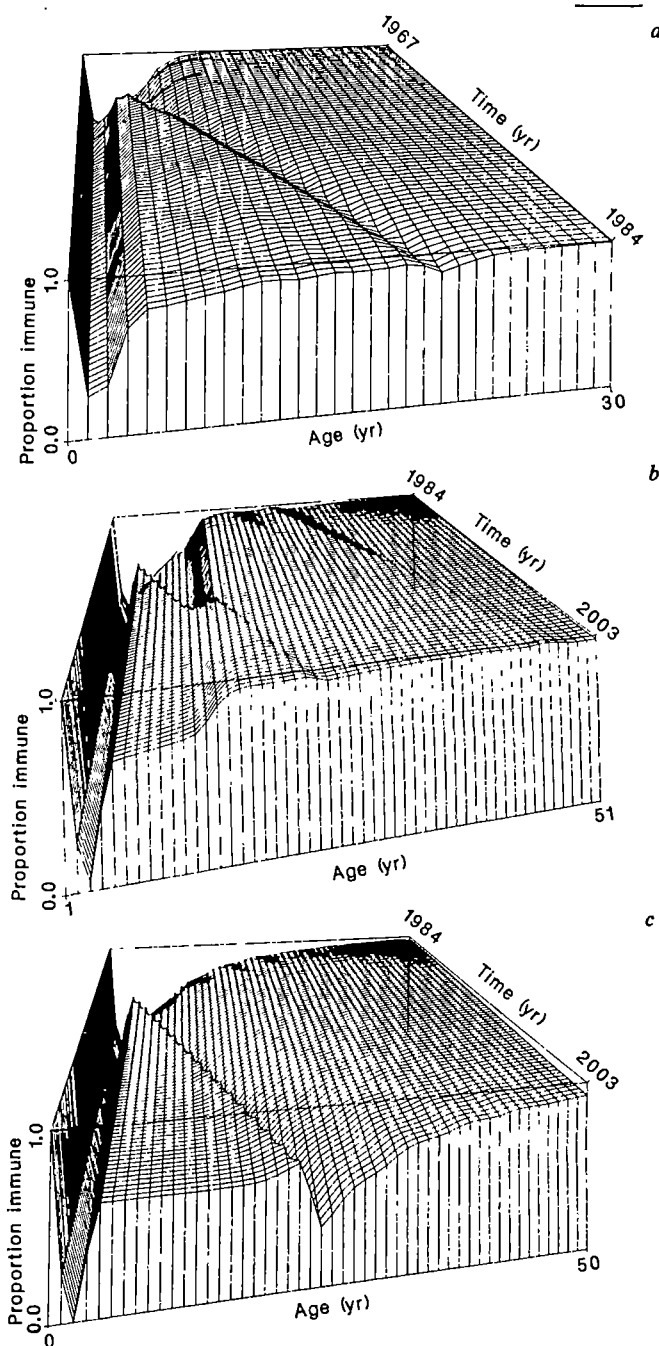


Fig. 4 a, Model predictions (equations (1)) of the impact of mass vaccination on the age-serological profiles for measles antibodies (the proportion in each age class who are immune either as a result of maternally derived protection (in the infants), recovery from natural infection, or vaccination) in England and Wales²⁵. Mass vaccination was introduced in year 1 (year 0 denotes the pre-vaccination profile, based on the age-specific forces of infection defined in Fig. 3a) and maintained for 16 yr with the age-specific vaccination rates recorded in England and Wales (mean age at vaccination ~2.2 yr; ~50–60% of each yearly cohort was immunized by age 5 yr). The age axis denotes the intervals 0, 0.5, 1.0, 2.5, ... etc. yr. Note how mass vaccination (in the age range 1–3 yr) acts to decrease the proportion seropositive in the older age classes (by reducing the overall force of infection within the community below the level in the pre-vaccination community). The valley of seronegativity running diagonally across the surface denotes the ageing of the last cohorts of young children to be excluded from mass immunization (see ref. 25 for further details). b, c, Similar to a but denoting model predictions of the impact of vaccination on the age-serological profiles for rubella antibodies over the period 1984–2030 in the United Kingdom in the male (c) and female (b) segments of the population. The basis of the predictions is described in ref. 67. In the period 1970–84 a single-stage UK policy was in operation (vaccination of teenage girls between the ages of 10–15 yr, with the observed vaccination rates for England and Wales). In 1985 a two-stage (UK-US) policy was begun (vaccination of teenage girls at 1984 rates, vaccination of 60% of 2-yr-old boys and girls) to be maintained up to 2030. Note how the introduction of the two-stage policy alters the level of herd immunity in both the male and female segments of the population. The age axis records the intervals 0, 0.5, 1.5, 2.5, ... 49.5 yr.

hence the creation of herd immunity may require repeated cohort immunization. Cost factors and other practical issues are therefore likely to be of great importance in developing countries when considering the benefits of mass vaccination versus mass chemotherapy.

Conclusions

Many difficulties surround the attainment of sufficient levels of herd immunity to eradicate common infections in developed and developing countries. Theory can define the level of vaccination coverage required for elimination, but success in practice depends on economic and motivational issues. Vaccines against protozoan and helminth parasites will undoubtedly be of importance in the future (certainly to the Western traveller to tropical regions, but less certainly to those who live in these areas), and research towards their development must remain a priority. This emphasis, however, should not distract attention from the importance of epidemiological research. Effective community-

wide control of many of the world's major infections is likely to depend on integrated approaches, involving some combination of selective or targeted vaccination and chemotherapy (directed towards those most predisposed to infection and morbidity), vector control and efforts to improve nutrition, hygiene, sanitation and education. Insights founded on an understanding of the population dynamics of infection will, therefore, be of great importance in any quantitative assessment of control policy. The recent convergence of mathematical theory and observation in epidemiology has created a powerful set of tools for the design and evaluation of community-based programmes of disease control, provided they are used sensibly. At present the potential value of these techniques is not widely appreciated.

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Continental rifts as a setting for regional metamorphism

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During very high-temperature/low-pressure Hercynian metamorphism in the Pyrenees the crust began to melt at ~12 km and stable isotopes show that it was flushed by circulating seawater to that depth. There is no evidence for crustal collision and the tectonic setting for this, and maybe all high-temperature/low-pressure metamorphism, is a zone of continental rifting.

RIFT valleys are familiar features of the Earth's surface. Some, such as the Rhine Graben, are active today and have associated earthquakes and volcanic activity; others, such as the Midland Valley of Scotland, may be recognized only by the presence of a down-faulted belt of younger rocks preserved in a more ancient terrane. Such rifts must have some expression deeper in the crust, but, at present, its nature is unknown, and there is no way of recognizing them in ancient and deeply eroded areas. Here we link continental rifting in a genetic association with high-temperature/low-pressure (high-*T*/low-*P*) regional metamorphism, an equally common crustal feature but one of uncertain tectonic setting. We argue that the transient physical conditions required for the metamorphism can probably be attained only during rifting; and that the geophysical signatures characteristic of the crust below an active rift can best be satisfied by rocks with the physical properties of those undergoing very high grade metamorphism. We support these conclusions with a detailed study of an exceptionally well exposed Hercynian metamorphic terrain in the eastern Pyrenees.

Eastern Pyrenees

In the Pyrenees, regional early Tertiary uplift has exposed an extensive Hercynian basement terrain (Fig. 1) including abundant granitoid intrusives and several condensed metamorphic sequences that imply exceptionally high thermal gradients^{1,2}. Subsequent Alpine metamorphism and deformation has been slight and is confined to the main fault zones. In between these faults, relatively pristine slices of basement have been differentially elevated and, in consequence, well-preserved Hercynian rocks from a variety of structural levels are often clearly exposed in high mountainous terrains. These range from Upper Carboniferous/Lower Permian sedimentary strata and volcanics,

through Lower Palaeozoic metasediments to granulites from deep crustal levels.

The Trois Seigneurs Massif (Fig. 1) is a thrust slice of Hercynian basement occurring to the north of the zone of maximum uplift. Here, a complete section from fossiliferous Silurian shales through andalusite and sillimanite schists to migmatites and a heterogeneous S-type granitoid is clearly exposed. A section through part of the metamorphic sequence is shown in Fig. 2. The lithologies in the sequence are dominantly pelitic but rare, thin carbonate bands are also present and provide a complimentary control on metamorphic grade. A variety of lines of petrological evidence³ indicate that the onset of melting in the field sequence (believed to correspond to the first appearance of migmatites) was at ~700 °C and at 3.0–3.5 kbar: this conclusion is supported by field and petrographic evidence (see below) and by experimental melting studies on the pelities carried out over a wide *P/T* range, with various water activities. Metamorphism and anatexis occurred under water-rich conditions with *a*_{H₂O} buffered externally near unity, leading to the generation of large quantities of granitic melt from the pelitic rocks³.

Metamorphism and melting were accompanied by deformation and the formation of flat-lying isoclinal folds with axial surfaces parallel to the isograds in all the higher grade rocks^{1,4,5}, but this deformation had mostly ceased before the metamorphic peak was reached because some of the prograde minerals overgrow these structures. Synmetamorphic leucogranite pods generated by partial melting of pelitic metasediment are commonly boudinaged and extended parallel to the isograds. Both of these extensional deformations have previously been attributed to doming by granitic diapirs⁴ but the boudinage (and possibly other deformational features) could equally well result from regional ductile extension of the metamorphic and granitic rocks. Similar metamorphic sequences are exposed elsewhere in the Pyrenees (for example, in the St Barthelemy Massif^{6,7}).

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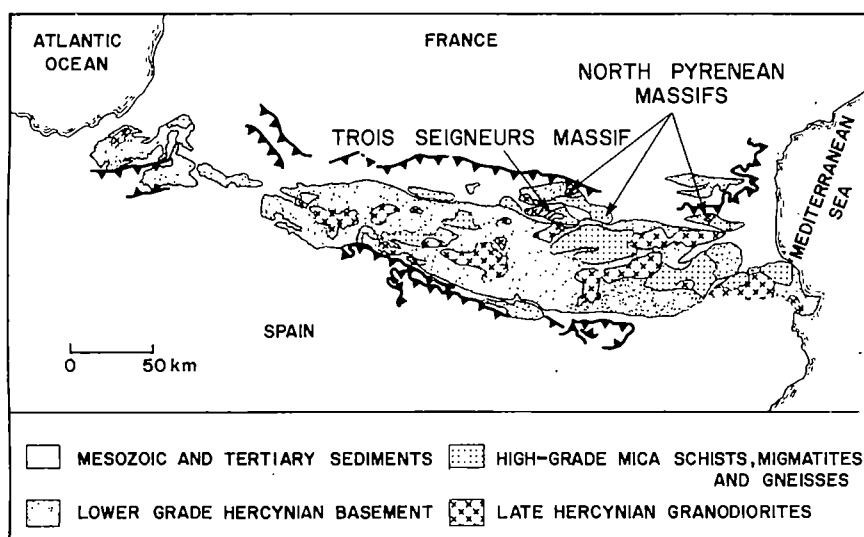
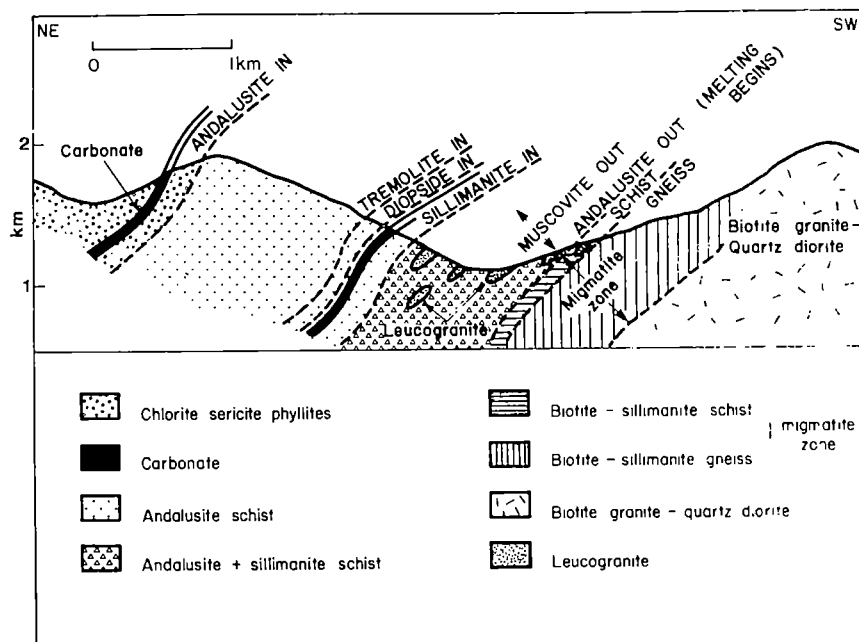


Fig. 1 Map showing the extent of Hercynian basement outcrop in the Pyrenees. The locations of the Trois Seigneurs Massif and other North Pyrenean Massifs are shown.

Fig. 2 Simplified cross-section through the Trois Seigneurs Massif showing the gradational transition from chlorite grade phyllites, through andalusite- and sillimanite-bearing mica schists to migmatites and a per-aluminous granitoid derived by partial melting and homogenization of the pelitic rocks. Note the proximity of low- and high-grade rocks.



Detailed mapping has established that little penetrative deformation has affected the metamorphic sequence since the Hercynian, and it is possible to estimate a thermal gradient for the upper part of the Hercynian crust by measuring the 'stratigraphic distance' (Fig. 2) across a series of isograd surfaces to which temperatures have been assigned. Absolute pressure for the onset of melting can independently be fixed at 3–3.5 kbar by a variety of constraints³, including the mineralogy of the metamorphic sequence (andalusite-sillimanite facies series, staurolite absent, garnet absent except at very Mn-rich compositions), the sequence of isograds (sillimanite in—muscovite out—melting begins), the fact that biotite is a stable phase throughout melting, and the use of biotite-cordierite geobarometry⁸. Having fixed these pressure limits, water activity is also constrained to be high because of the isograd sequence and because biotite persists throughout melting (see refs 3, 9). Where the bases of the high-grade metamorphic sequences are exposed they are seen to pass downwards through migmatite zones into anatectic granites containing disorientated fragments of the more refractory constituents of the former country rock.

The metamorphic reactions corresponding to the isograds documented in the field give a P/T array with a slope of $80\text{--}100\text{ }^{\circ}\text{C km}^{-1}$ for the Hercynian upper crust during metamorphism in the Trois Seigneurs Massif (Fig. 3). Similar metamorphic sequences in many other parts of the Pyrenees^{1,10,11} imply that exceptionally high gradients were developed on a regional scale. Note that slopes of such P/T arrays do not necessarily correspond to true crustal thermal gradients, insofar as the conditions corresponding to the P/T points in the array may have been attained at different times [see ref. 12]. Without a proper understanding of the processes controlling heat and cooling, the significance of such an array is ambiguous.

Hercynian crust during metamorphism

If the P/T array shown in Fig. 3 corresponds to a temperature gradient that existed within the upper part of the Hercynian crust, it could not have continued to depths ≥ 14 km without almost total melting of the crust below this depth, regardless of lithological composition or water content. A similar conclusion is reached by considering the average thermal gradient for the upper crust derived by joining any point in the array to a mean value for the surface temperature.

The crustal temperature distribution implied by these observations cannot represent a steady state: both the geological and geobarometric evidence indicate that the exceptionally high temperatures were not attained by burial, thereby leaving several alternatives. Heat could have been advected to the upper crust

by magma or other fluids from, say, the lower crust or the mantle^{13,14}. A mafic magma could, if present in sufficient quantity and emplaced at a suitable depth (say, 15 km), maintain for a limited period the conditions observed. Such an intrusion would, however, have to be a significant fraction of the crustal thickness (~ 10 km).

Alternatively, high temperatures in the upper crust could have been brought about by a change in the temperature at the base of the crust and thus an increased crustal conductive heat flow. An increase in the temperature at the base of the crust sufficient to give the observed upper crustal conditions, would inevitably also be high enough to melt the lower crust (for any reasonable composition, wet or dry) and in this case heat would be transferred advectively by the ascent of granitoid plutons [see for example, refs 4, 15].

Other evidence

Four other lines of evidence constrain the behaviour of the crust in the eastern Pyrenees during the metamorphic and deformational processes described above.

(1) Extensive granodioritic bodies (such as Mont Louis, Maladeta, and Querigut) have penetrated the whole area; they cut all other lithologies described above and develop contact metamorphic aureoles against them. They were, however, probably emplaced shortly after the cooling of the metamorphic

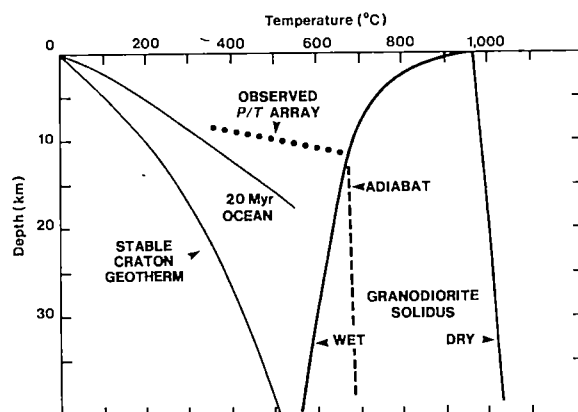


Fig. 3 Observed Trois Seigneurs P/T array compared with typical geotherms for a stable craton and 20-Myr-old oceanic crust. Anatexis of pelitic metasediment in the Trois Seigneurs migmatite zone occurred at $700\text{ }^{\circ}\text{C}$ and 3.0–3.5 kbar.

sequences (see below) and their isotopic and bulk chemical properties suggest that they were derived from the deeper crust^{16,17}. This shows that partial fusion of the Hercynian crust was not restricted to the anatectic zones described earlier. It is, therefore, possible, although on the grounds of timing not likely, that the ascent of the granodioritic magmas transported heat for upper crustal anatexis.

(2) There is local evidence for the incorporation of mafic material in the main, high level, anatectic zone. At higher structural levels mafics are virtually unknown. If the upper crust was heated by the intrusion of mafic magmas, they must mostly have ponded in the middle crust and risen little above that level.

(3) In various places in the eastern Pyrenees, fault-bounded slices of granulite and amphibolite facies gneisses are exposed (see refs 14, 18). The age of these bodies is not well established but in all probability their main metamorphic mineral assemblages are Hercynian, and in any case not younger. Different slices show equilibration temperatures around 700–850 °C and 3.5–7 kbar (ref. 14). Many of them also show evidence of small scale partial melting with development of leucosomes. If these rocks are Hercynian in age they show that the 'middle' crust (for a conventional crustal thickness) was close to its melting temperature at that time. The slices were emplaced to their present levels as a result of Tertiary deformation.

(4) Whereas the previous three considerations relate to the middle and deeper part of the crust during metamorphism, stable isotope studies can be used to constrain processes nearer the surface. Oxygen isotope studies were carried out on 95 rocks and minerals at different levels in the metamorphic sequence. It was shown¹⁹ that in the Trois Seigneurs Massif, the isotopic composition of oxygen at all exposed levels deeper than the andalusite-in isograd has been homogenized at $\delta^{18}\text{O}$ values of +11 to +13‰. Original values in pelitic lithologies would have been about +15‰. Material balance calculations suggest that isotopic compositions were homogenized by contact with a circulating volume of aqueous fluid with total mass probably >30% of the mass of rock affected¹⁹. This means that the fluid is almost certainly of external origin. Deuterium/hydrogen studies on the same material indicate that the fluid was rich in D with a δD of –10 to –15‰. This corresponds to a probable value for late Carboniferous seawater. These considerations suggest, therefore, that the metamorphic-anatectic sequence and the overlying sediments were flushed by circulating seawater during or before the metamorphic maximum.

Pyrenees in the late Palaeozoic

We now examine some broader aspects of the geology that relate to the regional setting of the Hercynian metamorphism in the Pyrenees to see to what extent they are compatible with rifting.

Within the Pyrenees the Palaeozoic sequence that subsequently underwent Hercynian metamorphism extends from the Cambrian to various horizons in the Carboniferous and comprised shales (dominantly), impure sandstones, carbonates and locally quartzites. There is relatively good palaeontological control of ages and the Devonian and Carboniferous are effectively unmetamorphosed. Conversely the highest grades of metamorphism are restricted to the Cambro-Ordovician rocks. The youngest strata in the Trois Seigneurs area are Silurian, but nearby in the Axial Zone the Westphalian (298–315 Myr) is present. Note that the cooling ages of the Trois Seigneurs granodiorite (post-metamorphic) and probably also the metamorphic sequence are 315 ± 10 Myr. This implies that metamorphism of the lower parts of the sedimentary pile was taking place at the same time as deposition was continuing at the surface. The metamorphic maximum was shortly followed by the ascent of granodiorite bodies that show discordant relations with both metamorphosed and unmetamorphosed Palaeozoic. However, the isotopic composition of oxygen in the Trois Seigneurs granodiorite is undisturbed and so the high level hydrothermal circulation that affected the metasedimentary sequence had ceased before its emplacement.

This main sequence of Palaeozoic deposits in the Pyrenees is terminated by an unconformity at which conglomerates, volcanics and continental deposits of Permian or even Stephanian age commonly overlie Westphalian rocks. The time interval represented by the unconformity varies from place to place.

Ophiolitic rocks have not been reported from the Hercynian Pyrenees nor is there any other evidence of involvement of oceanic rock types. There has, however, been substantial Mesozoic and Tertiary movement of Iberia with respect to France and the early Mesozoic position of Iberia cannot be established with confidence. There is even less control on its relative position in the late Palaeozoic, and therefore it is not clear which areas outside the Pyrenees themselves comprised their Palaeozoic regional setting. However, there does seem to be a direct connection between the Carboniferous of the western Pyrenees and that of the eastern Cantabrian mountains of northern Spain. Upper Carboniferous sedimentation is there characterized by the development of locally very thick successions in small but deep basins. Strikingly angular unconformities punctuate the Upper Carboniferous from the Lower Westphalian to the Lower Autunian. The main sedimentary characteristics are high sediment input, very thick successions (up to 6,000 m locally with a total thickness >15,000 m), rapid changes of facies, both vertically and laterally, and indications of contemporaneous gravity driven movement. These features led Reading²⁰ to propose that they represented deposition in extensional regions developed within a strike-slip orogenic belt, movement probably being accommodated on NW-SE and WNW-ESE trending dextral faults, a direction that is compatible with the regional movement of Africa with respect to Europe and North America at that time¹².

Carboniferous rocks are not exposed immediately north of the Pyrenees in the Aquitaine basin, but, in any case, Iberia was probably closer to Brittany and south-west England at that time. Arthaud and Matte²¹ have summarized the evidence that during Carboniferous and Permian time much of southern and central Europe was affected by rifting.

The setting of Hercynian metamorphism in the Pyrenees is poorly constrained. There is no evidence of continental collision or ocean closure. There was, however, contemporaneous rifting outside the area, and possibly strike-slip movement²¹. Metamorphism seems to have occurred towards the end of a prolonged period of basin subsidence and deposition. Both the evidence of deep circulation of seawater through the sedimentary pile and geochronological evidence suggest that the region was below sea level and still receiving sediment while metamorphism was going on at depth.

A rifting setting

The sequence and timing of events documented above is quite different from that recorded from areas such as the Alps and Himalayas where metamorphism has followed crustal collision and tectonic burial. Metamorphism in a collision setting is typically associated with: (1) nappe-type folding and/or thrusting on a regional scale; (2) a delay of some tens of millions of years between collision (and thus the termination of sedimentation) and the metamorphic maximum; (3) the early development of high-pressure mineral parageneses; (4) the common, but not ubiquitous, preservation of lithologies derived from former oceans. None of these features is present in the Hercynian of the Pyrenees.

Active rifts have been studied in many parts of the world^{22,23}. Some, such as the East African Rift and the Rhine Graben seem to be purely extensional features²⁴. Others, such as the Salton Sea and the Dead Sea, are extensional features associated with major strike-slip movements. Typically they are seismically active, topographically low and continue to receive sediment throughout their active lives. Some such as the Central and Viking grabens of the North Sea are localized zones of extension within a regional extensional basin. The rifts commonly have associated volcanism, hydrothermal activity, and high, but variable, surface heat flow.

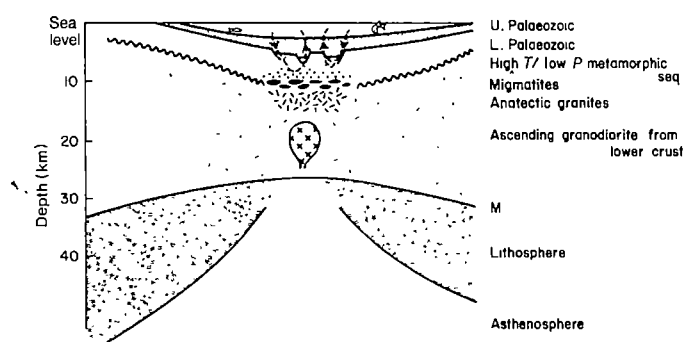


Fig. 4 Schematic tectonic setting for the Hercynian high- T /low- P metamorphism in the Pyrenees. Seawater circulates within the Palaeozoic sedimentary pile, towards the bottom of which metamorphism and large-scale anatexis is occurring at 700 °C and 10–12 km depth. The lower crust is heated to very high temperatures by hot, upwelling asthenosphere, and large bodies of granodiorite magma are generated within this deeper melting zone.

Among the most important geophysical features of presently active rifts are the anomalously low crustal seismic velocities recorded beneath them, and the anomalous attenuation of seismic waves passing through them. Both features may be explained by abnormally high crustal temperatures leading to varying degrees of partial melting. Indeed it is difficult to find any plausible alternative.

Probably the most appropriate modern-day analogue for the processes we propose is to be found in the Salton Sea area of southern California²⁵. The regional tectonic setting is one of strike-slip and extension, associated with the southern continuation of the San Andreas fault system and the extensional regime of the Gulf of California spreading centre. In the latter area, the regional heat flow is $\sim 130 \text{ mW m}^{-2}$ but in the Salton Sea area and some of the associated geothermal fields the heat flow is much higher; temperature gradients of $>150 \text{ }^{\circ}\text{C km}^{-1}$ are measured over the upper 2 km. Locally, there are substantial surface flows of hot water and the drilled sections are, and have been, clearly flushed with hot brines. Muffler and White²⁵ document a series of low-pressure/high-temperature prograde metamorphic reactions from cores in the drilled section.

Although the other rifts, such as the Rhine Graben and the Rio Grande Rift, are associated with high heat flow and local magmatic activity the hydrothermal surface manifestations are less extreme than those observed in the Salton Sea. Seismic reflection work²⁶ indicates the presence of a magma body at 19–20 km depth under Socorro in New Mexico in the southern part of the Rio Grande Rift. There is no way of knowing its temperature or composition but it could be sufficiently hot to

bring about partial melting in the crust immediately above it, providing an analogue for the Trois Seigneurs migmatite. Both P - and S -wave velocities are lower under the Rio Grande Rift than in the regions to either side²⁷.

Discussion and conclusions

The relationship between thermal and mechanical phenomena in rift zones is incompletely understood. It is, however, plausible that the maximum perturbations of crustal temperature should occur where the lithospheric thinning is greatest. Stretching may extend uniformly along a rift or may occur heterogeneously beneath a series of staggered, *en echelon* pull-apart basins. In either case, there may be mafic magmatic activity and the temperature at the base of the crust is elevated as a consequence of lithospheric thinning (Fig. 4); the possible importance of such zones for metamorphic processes has been noted by others^{28–30}. Such zones seem to offer the best possibility for attaining the upper crustal P/T conditions described earlier, and a very hot and partially molten middle and lower crust is suggested both by the attenuation and velocity reduction observed across active rifts today, and by the migmatites and the granulite slices from the Hercynian Pyrenees.

We conclude that the Hercynian 'orogeny' in the Pyrenees was a rifting event, possibly associated with strike-slip motion, that affected a continuously subsiding Palaeozoic Basin; that the deformation and metamorphism occurred well below sea level and that seawater was circulating into the crust to depths of 10 km or more during the process. Such an interpretation is consistent with the regional geology of western Europe at that time.

Rifts probably provide a wide spectrum of crustal environments, but virtually all seem to lead to abnormally high crustal temperatures and thus the possibility of high-temperature/low-pressure metamorphic conditions. Whether seawater flushing occurred in any particular instance, would depend on the degree of local extension (and thus subsidence) and access to the sea. The high-temperature/low-pressure elements in the so-called paired metamorphic belts of the Pacific region probably develop by rifting and extension within island arcs. The tectonic interpretation of high temperature belts in old crustal terrains must be reconsidered if the views presented here are correct.

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Topology of signal recognition particle receptor in endoplasmic reticulum membrane

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The signal recognition particle (SRP) receptor is an integral membrane protein of the endoplasmic reticulum which, in conjunction with SRP, ensures the correct targeting of nascent secretory proteins to this membrane system. From the complementary DNA sequence we have deduced the complete primary structure of the SRP receptor and established that its amino-terminal region is anchored in the membrane. The anchor fragment and the cytoplasmic fragment contribute jointly to a functionally important region which is highly charged and may function in nucleic acid binding.

WE have recently reviewed the function of the signal recognition particle (SRP) and the SRP receptor in the translocation of secretory and lysosomal proteins across the endoplasmic reticulum (ER) membrane, as well as their function in the integration of certain classes of integral membrane proteins into this membrane¹. SRP is thought to recognize the signal peptide on the nascent proteins and arrest their translation in the cytoplasmic space. On interaction of the elongation-arrested ribosome with the ER membrane, which involves a direct interaction of the ribosome-bound SRP with the SRP receptor in the ER membrane, the elongation arrest is released. The SRP receptor is required for protein translocation even in the absence of elongation-arrest², and it thus seems to function primarily to target the SRP-associated nascent polypeptides to the ER membrane. Thereafter, a functional ribosome-membrane junction is established that allows the translocation of the growing polypeptide chain across the ER membrane by a mechanism that is, as yet, poorly understood.

Proteolytic dissection of SRP receptor

Using *in vitro* protein translocation assays as the principal tool, two different approaches³⁻⁸ eventually converged in the discovery and purification of the SRP receptor (or docking protein), as the first—and so far only—integral membrane protein known to play an essential part in this process of protein targeting. Proteolytic dissection of the SRP receptor gave a preliminary picture of its disposition in the ER membrane⁸. The bulk of its mass constitutes a cytoplasmic domain of relative molecular mass (M_r) 52,000 (52K) (see below; by SDS-polyacrylamide gel electrophoresis (PAGE) its size was previously estimated to be 60K) that can be severed from the membrane by a variety of proteases, and retains the ability to reconstitute the activity of proteolysed membranes. By itself, the cytoplasmic fragment—in contrast to the detergent-solubilized intact SRP receptor^{7,8}—is unable to release the SRP-induced arrest of presecretory proteins^{2,6}.

This finding raised the question of whether the 52K SRP receptor fragment still retains affinity for SRP. To directly examine this possibility, we mixed the 52K SRP receptor fragment with a fraction enriched in SRP receptor (Fig. 1, lane 1). We then applied this mixture to an SRP affinity column and monitored the elution of both the 52K SRP receptor fragment and the SRP receptor using Western blot analysis. Intact SRP receptor bound to the SRP affinity column and could be eluted with an increase in the magnesium concentration, as described previously⁹ (Fig. 1, lane 3). In contrast, the 52K fragment was only slightly retarded in the affinity column and was quantitatively recovered in the pooled flow-through and wash fraction (Fig. 1, lane 2). We conclude that the affinity of the 52K receptor fragment for SRP is greatly decreased compared with the intact receptor. Taken together, these findings suggest that the part of

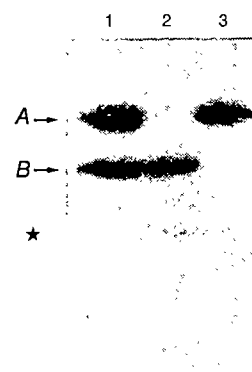


Fig. 1 Chromatography of the SRP receptor and the 52K cytoplasmic fragment on SRP-Sepharose. Lane 1, load fraction; lane 2, combined flow-through and wash fractions; lane 3, column eluate. Arrows indicate the positions of the intact SRP receptor (A) and the 52K cytoplasmic fragment band (B), respectively. The asterisk denotes an additional minor breakdown product (~45K) that was contained in the 52K cytoplasmic fragment fraction.

Methods. SRP receptor was partially purified from canine pancreas following the procedure described elsewhere⁹, excluding affinity chromatography on SRP-Sepharose. The 52K cytoplasmic fragment was purified as described elsewhere². Both fractions were mixed to yield 600 μ l of a solution containing 350 ng SRP receptor and 300 ng 52K cytoplasmic fragment in buffer A (50 mM triethanolamine/HOAc pH 7.5, 20 mM KOAc, 5 mM Mg(OAc)₂, 250 mM sucrose, 0.5% octaethyleneglycol-mono-*n*-dodecyl ether (Nikkol; Nikko Chem. Corp., Tokyo, Japan), 1 mM dithiothreitol) and 15 mM NaH₂PO₄. The mixture was applied to a 150- μ l SRP-Sepharose column (containing 40 μ g coupled SRP). The binding capacity of this column was empirically determined to be in excess of 1 μ g SRP receptor bound per 150 μ l of resin. The column was washed with 600 μ l of buffer A containing 50 mM KOAc and eluted with 450 μ l of buffer A containing 10 mM KOAc and 25 mM Mg(OAc)₂. Fractions corresponding to 10% of the load were precipitated with trichloroacetic acid and subjected to SDS-PAGE. The gel was blotted onto nitrocellulose. SRP receptor and the 52K cytoplasmic fragment were detected by incubation with a monoclonal antibody against SRP receptor (V. Rath and P.W., to be described elsewhere) followed by incubation with iodinated second antibody and autoradiography²¹.

the SRP receptor that remains membrane-associated after proteolysis does not only contain sequences required to anchor it in the ER membrane, but must also contribute to a functionally important domain of the intact receptor.

Protein sequencing

Clearly, knowledge of the primary sequence of the SRP receptor would help our understanding of its structural organization. We therefore purified SRP receptor and the 52K SRP receptor

A met leu asp phe phe thr ile phe thr lys gly gly leu val leu trp

5' ATG CTC GAC TTC TTC ACC ATC TTC ACC AAG GGC GGC CTC GTG CTC TGG 3'

3' TAC GAG CTG AAG AAG TGG TAG AAG TGG TTC CCG CCG GAG CAC GAG ACC 5'

B

λ SR 1

λ SR 31

λ SR 32

λ SR 50

λ SR 63

100 bp

S SP S S A SP A S P H P H A P S S P S

C

GGGAGCTCAGGCCGATCGCCGCCGCTTCTGCTGCCGCC 1 10

met leu asp phe phe thr ile phe ser lys gly gly leu val leu trp cys phe gln

ATG CTC GAC TTC TTC ACC ATT TTC TCC AAG GGC GGC CTT GTG CTC TCG TGC TTC CAG 98

20 30 40

gly val ser asp ser cys thr gly pro val asn ala leu ile arg ser val leu leu gln arg gly gly asn asn ser phe thr his

GGC GTG AGC GAC TCC TGC ACC GGC CCC GTT AAT GCG TTG ATT CGA TCT GTG CTG CTG CAG GAA AGG GGA GGT AAC AAC TCC TTC ACC CAT 188

50 60 70

glu ala leu thr leu lys tyr lys leu asp asn gln phe glu leu val phe val val gly phe gln lys ile leu thr leu thr tyr val

GAG GCG CTC ACA CTC AAG TAT AAA CTG GAC AAC CAG CTC GAG CTG GTG TTC GTG GTC GGT TTT CAG AAG ATC CTA ACC CTG ACG TAC GTA 278

80 90 100

asp lys leu ile asp asp val his arg leu phe arg asp lys tyr arg thr glu ile gln gln gln ser ala leu ser leu leu asp gly

GAC AAG TTG ATA GAT GAT GTG CAT CCG CTG TTT CGA GAC AAG TAC CGC ACA GAG ATC CAA CAG CAA AGT GCC TTA AGT CTA TTG AAC GCG 368

110 120 130

thr phe asp phe gln asn asp phe leu arg leu leu arg glu arg glu glu ser ser lys ile arg ala pro thr thr met lys lys phe

ACT TTT GAT TTC CAG AAT GAC TTC CTG CCG CTC CTT CGC GAA CGA GAG GAG AGC AGT AAG ATC CGT GCT CCC ACT ACC ATG AAG AAA TTC 450

140 150 160

glu asp ser glu lys ala lys lys pro val arg ser met ile glu thr arg gly glu lys pro lys glu lys ala lys asn ser lys lys

GAA GAT TCT GAA AAG GCC AAG AAA CTT GGT AAG TCC ATG TCC ATG ATT AGA CGG GGT GAA AAG CCC AAG GAA GAA GCA AAG AAC AGC AAA AAA 548

170 180 190

lys gly ala lys lys glu ser ser asp gly pro leu ala thr gly lys ala val pro ala gly lys ser glu pro ala gly pro glu

AAG GGG GCC AAG AAG GAG AGC TCT GAT GGC CCT TTG GCT ACG GGC AAA GCA GTT CCT GCT GAA AAG TCA GGT CTC CCA GCG GGG CCA CAG 630

200 210 220

asn gly val glu leu ser lys glu glu leu ile arg arg lys arg glu glu phe ile gln lys his gly arg gly leu glu lys ser ser

AAC GGG GTA GAA CTT TCC AAA GAG GAG CTA ATA CGC AAG AAG CCG GAG GAG TTC ATT CAG AAG CAT GGG AGA GGT CTC GAG AAG TCC AGC 720

230 240 250

lys ser thr lys ser asp ala pro lys glu lys gly lys ala pro arg val thr ala leu gly gly cys ala asn lys glu val leu

AAG TCC ACG AAA TCA GAT GCT CCG AAG GAG AAG GGC AAG AAA GCA CCC CGG GTG TGG GCA CTA GGT GGC TGT GCT AAC AAG GAA CTT TTG 818

260 270 280

asp tyr ser ala pro thr thr asn gly ala pro asp ala pro pro glu asp ile asn leu ile arg gly thr gly pro gly gly gln

GAT TAT AGC CTT CCC ACC ACC AAT GGA GCC CCT GAG GCT GCC CGC GCT GAG GAC ATC AAC TTG ATT CGA GGG ACT GGG CTT GCG GGG CAG 900

290 300 310

leu gln asp leu asp cys ser ser ser asp asp glu glu thr ala gln asn ala ser lys pro ser ala thr lys gly thr leu gly gly

CTT CAG GAT CTG GAC TGC AGC AGC TCA GAT GAT GAA GAG ACC GCA CAA AAT GCC AGC AAA CCC AGT GCT ACC AAG GGA ACT CTC GGT GGC 998

320 330 340

met phe gly met leu lys gly leu val gly ser lys ser leu ser arg glu asp met glu ser val leu asp lys met arg asp his leu

ATG TTT GGG ATG CTG AAG GGC CTT GTG GGG TCC AAG AAG TGT GGT GAT GCT GGT AAG GAA TCG GTG CTG AAG AAG ATG CGT GAT CAT CTC 1088

350 360 370

ile ala lys asn val ala ala asp ile ala val gln leu cys glu ser val ala asn lys leu glu gly lys val met gly thr phe ser

ATT GCT AAG AAT GTG GCA GCA GAT ATT GCA GTG CAG CTC TGT GAA TCT GTG GCC AAC AAG CTG GAG GGG AAG GTG ATG GGG ACA TTC AGC 1178

380 390 400

thr val thr ser thr val lys gln ala leu gln glu ser leu val gln ile leu gln pro gln arg arg val asp met leu arg asp ile

ACG GTG ACT TCC ACA GTA AAG CAA GCC CTG CAA GAG TCC CTG GTG CAG ATT CTG CAG CCA CAG CGT GCT GTA GAC ATG CTC CCA GAT ATT 1268

410 420 430

met asp ala gln arg his gln arg pro tyr val val thr phe cys gly val asn gly val gly lys thr thr asn leu ala lys ile ser

ATG GAG GCC CAG CGT CAT CAG GCG CTT TAT GTT CTC ACT TCT TGT GGT ATT AAG GGA GTG GGG AAG TCT ACT AAT CTT GCC AAG ATT TCC 1358

440 450 460

phe trp leu leu glu asn gly phe ser val leu ile ala ala cys asp thr phe arg ala gly ala val glu his val arg thr his thr

TTC TGG CTG CTA CAG AAT GGC TTC AGT GTC CTC ATT GCT GCC TGT GAC ACA TTT CGT GCT GGG GCC GTG GAG CAC GCG CCA CAC ACC 1448

470 480 490

arg arg leu ser ala leu his pro pro glu lys his ala gly pro thr met val gln leu phe glu lys gly tyr gly lys asp ala ala

CGG CCG CTG AGT GCC CTA CAC CCC CCA GAG AAG CAC GCT GGC CCG ACT ATG CTG CAG TTG TTT GAA AAG GGC TAC GGC AAG GAC GCT CCA 1538

500 510 520

gly ile ala met glu ala ile ala phe ala arg asn gln gly phe asp val val leu val asp thr ala gly met gln asp asn ala

GCG ATT GCC ATG GAA CCG ATT GCC TTT CCA CGT AAC CAA GGC TTT GAT GTG GTG CTG GTG GAC ACA GCT GGC CCG ATG CAA CAG AAT GCC 1628

530 540 550

pro leu met thr ala leu ala lys leu ile thr val asn thr pro asp leu val leu phe val gly glu ala leu val gly asn glu ala

CCT CTC ATG ACT GCC CTG GCT AAG CTC ATT ACT GTC AAC ACA CCC GAC TTG GTG CTG TTT GTG GGG GAG GCC TTA GTA GGC AAT GAG GCC 1718

560 570 580

val asp gln leu val lys phe asn arg ala leu ala asp his ser met ala gln thr pro arg leu ile asp gly ile val leu thr lys

GTG GAG CAG CTG GTC AAG TTC AAC AGA GCC TTG GCT GAC CAT TCT ATG CCT CAA ACA CCT GCG CTC ATT GAT GGC ATT GTC CTT ACC AAA 1808

590 600 610

gln asp thr ile asp asp lys val gly ala ile ser met thr tyr ile thr ser lys pro ile val phe val gly thr gln thr

TTT GAT ACC ATT GAT GAG AAG GTG GGA GCT GCT ATT TCT ATG ACC TAC ATC ACG AGC AAA CCC ATC GTC TTT GTG GGC ACT GGC CAG ACC 1898

620 630 638

tyr cys asp leu arg ser leu asn ala lys ala val val ala ala leu met lys ala OC

TAC TGT GAC CTA CGC AGC CTC AAT GCC AAG GCT GTG GTG GCT GCC CTC ATC AAG GCT TAA CATGGCTCTTGCCCAAAACCAATCACTGCTCCCCCA 1998

GCCCCCTCTCTGTATCAAGAAATGTGCTTTAGAATACGTGAGTGCTTCTCTCAGTGATGATACAGAGGCAGAGGGAGTCTTAGGGGCCCTGCAGTCTCCCCCTCCCCACTCTCTG 2118

GCACGCCAGCCCTCCACGCTGCAAGGGCTTAATCATGTTCACAGCTCCACTCCATCCACCCGCTGCTCCGACAGTCATCTCTCTACCTACGAGCTCTCTCTCTCTGCTTACACACTCAGTC 2230

CTATTACAGCTTTGACCAACGGTTGGAAGATCAACACACAGCTTTGCTAAATTCGACCGGTACACGGGATGTCGAGCACAATGAGCAGCTCGAGACTTCCAGAGCCAGCTCTGGGTAT 2350

CCCTTGATCCACAGCTTTCAGGCCCCCTGGGGCAGGATGATACCCACCACACTATGGCAGTGATGCGCTGTGCCAATTGCTGCTAATCTCTATGATGACCCCATGCCACCTGTTTCT 2478

CAAGCATTAGCTAGGCCAGAAATGATTGTGTAAGAAATGAACAAAGGAGTCTCTGCTTCCCTCAAAAATAAGAGGAGCTGAGCTGTCAGTGTGCAATGGGCTTCTACGCCCTCCAACTCT 2590

TCCCTGCTCCCAAGATTCAGATGTAAAGCTTTGCATGTTCTCTTCTTCCGAGAGAGAACAACCTGTCAAAAGGGTATACAAAGTTGCCCTCTCCCCACCCCACTTCCACTGAGTGAT 2710

TGCGCTTTCTCTCTCTCTCTCTCTACAGTGCCCTGGTAGACAAGTGCTACAGTTGAAGAATACAAACCTCTGTTAAGACTGTGCTGTGATGCTGTTATACAGATGTGCTATTAGTGCA 2830

TAAAGTGAAGGCTGTCTGCCAGAGAAATACATAAAATATATAAGATATACATTTTCATAAGTAAAGAAAAAAGAAAAAAGAAAAA 2931

above), (2) nucleotide 1,403 (T in λ SR31 and λ SR63, C in λ SR32; a conservative change) and (3) one of the eight nucleotides (G in λ SR31 and λ SR63, C in λ SR32) which was found to be deleted in λ SR32 and λ SR63. We attribute these latter discrepancies to errors of reverse transcriptase during the cDNA synthesis. The triangles in the restriction map indicate the beginning and end of the cDNA sequence used for the synthesis of the complementary DNA and predicted amino-acid sequence of the SRP receptor. Linker sequences have been omitted. The amino-terminal of the intact receptor (a) and the cytoplasmic fragment (b) are underlined. The region preceding Met 1 translates into Leu-Pro-Ala-Ala-Ala (-1). The polyadenylation signal, AATAAA, is boxed.

fragment from canine pancreas as described previously^{2,7}. Following preparative SDS-PAGE as an additional purification step, the proteins were eluted electrophoretically from the gels and subjected directly to sequence analysis on a gas-phase sequencer. Neither of the polypeptides appeared to be blocked at their amino termini and we were able to determine the first 24 amino acids of the SRP receptor and the first 10 amino acids of the 52K SRP receptor fragment (see Table 1). From these data, we concluded first that the SRP receptor and the 52K SRP receptor fragment have different amino termini, and second that the 52K SRP receptor fragment has a unique sequence which indicates that elastase—the protease used to sever this domain from the membrane—cleaves the SRP receptor in a unique position.

cDNA cloning

Knowledge of the partial primary sequences of these polypeptides allowed us to identify cDNA clones of the SRP receptor. According to codon usage frequencies¹⁰, we designed a single 48-nucleotide(nt)-long double-stranded DNA probe coding for the first 16 amino acids of the receptor (Fig. 2A) and used it to screen a cDNA library in phage λ gt10, constructed by oligo(dT)-priming of canine pancreatic poly(A)⁺ RNA (see Fig. 2 legend). From the library (containing ~500,000 clones), we isolated one recombinant phage (λ SR1, Fig. 2) that gave a strong hybridization signal and which contained an insert of 805 base pairs (bp). Re-screening of the library with this cDNA insert (see Fig. 2 legend for experimental details) did not yield any new cDNA clones, indicating that the isolated clone was the only

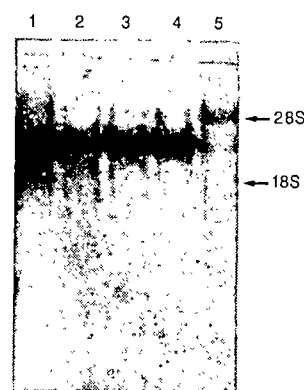


Fig. 3 Northern blot analysis of poly(A)⁺ RNA from MDCK cells (lane 1), canine kidney (lane 2), liver (lane 3), testis (lane 4) and pancreas (lane 5).

Methods. Total cellular RNA was prepared by the guanidine monothiocyanate/LiCl precipitation method²⁴ and selected on oligo(dT)-cellulose²⁵; 3.3 μ g (lanes 1–4) or 6 μ g (lane 5) of RNA was separated in a 1.2% agarose/formaldehyde gel²⁵ and transferred directly to nitrocellulose. Hybridization conditions were exactly as described in Fig. 2 legend using the insert of clone λ SR31 as probe (5×10^8 c.p.m. μ g⁻¹, 1.5×10^6 c.p.m. ml⁻¹). The film was exposed for 3 days. The two additional bands in lane 5 are probably due to nonspecific interaction of the probe with 18S and 28S ribosomal RNA; the relative proportion of these RNA species as visualized by ethidium bromide staining was higher in the pancreatic preparation than in the other lanes.

Table 1 Sequence analysis of the SRP receptor and the 52K SRP receptor fragment

Cycle	SRP receptor		52K cytoplasmic fragment	
	Amino acid	Yield (pmol)	Amino acid	Yield (pmol)
1	Met	194	Met	52
2	Leu	198	Ile	49
3	Asp	166	Glu	32
4	Phe	135	Thr	Not quantitated
5	Phe	180	Arg	27
6	Thr	48	Gly	31
7	Ile	138	Glu	25
8	Phe	130	Lys	33
9	Ser	22	Pro	11
10	Lys	61	Lys	27
11	Gly	67		
12	Gly	75		
13	Leu	96		
14	Val	88		
15	Leu	80		
16	Trp	12		
17	Not detected			
18	Phe	45		
19	Gln	16		
20	Gly	38		
21	Val	19		
22	Ser	5		
23	Asp	Very low		
24	Ser	4		

Both proteins were purified from canine pancreas as previously described^{2,9}. After preparative PAGE in SDS using a 7–11% gradient gel and electroelution²², the samples were applied directly to sequencer filters precycled with 1.5 mg of Polybrene. Sequence analysis was performed in a gas phase sequencer (Applied Biosystems model 470 A). The phenylthiohydantoin amino acids were separated using a Waters HPLC system equipped with a Rainin Microsorb C8 column (0.46 \times 25 cm) heated to 45 °C in a gradient of solvent I [0.25 M NaH₂PO₄ pH 4.6/CH₃CN/H₂O (10/18/72 by volume)] to solvent II [H₂O/CN₃CN (70/30)]. Initial yields were 88% and 40%, average repetitive yields amounted to 94% and 93% for SRP receptor and 52K cytoplasmic fragment, respectively. Protein determinations were performed by densitometric scanning of Coomassie blue stained bands in analytical SDS polyacrylamide gels using bovine serum albumin as a standard.

one containing these SRP receptor sequences. We therefore constructed further cDNA libraries from poly(A)⁺ RNA from MDCK (Madin Darby canine kidney) cells. Screening of these libraries (containing 500,000 recombinant clones) yielded four additional overlapping clones (λ SR31, 32, 50 and 63; schematically described in Fig. 2B) which cover the complete coding and 3'-untranslated sequence of the SRP receptor (see below).

We estimate that the SRP receptor constitutes only 0.1% of the protein contained in a rough ER cell fraction from pancreas. This low abundance, together with the fact that pancreatic messenger RNA largely encodes secretory and not cellular 'household' proteins, could account for the scarcity of SRP receptor sequences in this library. Indeed, in a Northern analysis of poly(A)⁺ RNA isolated from different canine tissues (Fig. 3), the relative abundance of SRP receptor transcripts was substantially higher in tissues with lower secretory activity. The best signal was obtained with RNA from MDCK tissue culture cells, presumably because a rapidly dividing cell is forced to constantly proliferate its ER membrane together with other vital cell functions.

Protein primary sequence

Analysis of the cloned cDNA sequence (2,931 bp) shows a single open reading frame that starts close to the 5' end. This reading frame contains a precise match with the amino-terminal amino-acid sequence of the SRP receptor extending beyond the amino acids used to design the oligonucleotide probe (Table 1, Fig. 2C). The predicted M_r of the encoded protein is 69,684, in good agreement with its apparent M_r of 72K, as estimated previously by SDS-PAGE⁷. Amino-acid analysis of the purified protein yields values close or identical to those derived from its predicted primary structure (data not shown). The cloned DNA extends for 41 nucleotides to the 5' side of the sequence coding for the amino terminus of the SRP receptor. Because this sequence does not contain an in-frame stop codon, we cannot formally exclude the possibility that the SRP receptor could be encoded as a precursor containing a cleavable signal sequence preceding Met 1. However, translation of these nucleotides (see legend to Fig. 2C) does not result in an amino-acid sequence resembling that commonly found in signal peptides¹¹. Therefore, it is possible that—as is the case for other integral membrane proteins (see ref. 12)—the information required for its integra-

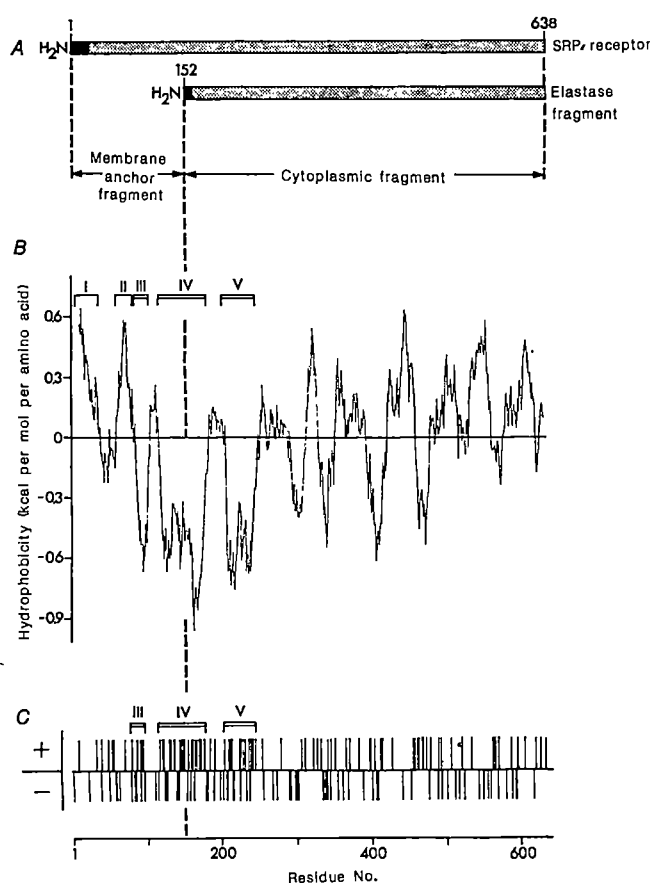


Fig. 4 Physical characteristics predicted from the SRP receptor primary structure. **A**, The locations of the membrane-anchoring fragment and the cytoplasmic domain extending to the carboxy terminus are indicated. Solid boxes mark the regions for which protein sequence data have been obtained; the vertical dashed line indicates the elastase cleavage site. **B**, Hydrophobicity values³³ were averaged for windows of 14 residues and are given as kcal per mol per amino acid of average free energy for the transfer from a hydrophobic to a hydrophilic environment. Regions I and II (amino acids 1–22 and 64–79, respectively) are candidates for sequence stretches interacting with the lipid bilayer. Clusters of charged residues are marked III (amino acids 84–97), IV (129–175) and V (205–243). **C**, Charge distribution along the SRP receptor sequence. All histidine, arginine and lysine residues were assumed to be positively charged (upward-projecting lines) and glutamic and aspartic acid residues to be negatively charged (downward-projecting lines).

tion into the membrane is encoded in an uncleaved signal sequence which in the SRP receptor would be contained in the amino-terminal portion of the protein (see below). The nucleotide context of the presumptive initiating ATG codon (GXXATGY) is also found in other eukaryotic mRNAs¹³, although it diverges from the most commonly found consensus sequence, AXXATGG¹⁴.

The SRP receptor and the 52K cytoplasmic fragment were purified by different methods and monitored using different activity assays^{2,9}. Sequence alignment of the amino-terminal region of the 52K SRP receptor fragment reveals that the fragment begins with Met 152 of the intact receptor; this provides independent confirmation of the identity of the cloned sequence as SRP receptor cDNA. From the position of the amino terminus of the 52K cytoplasmic fragment within the sequence, we conclude that the amino-terminal 151 amino acids (calculated M_r 17.5K) comprise the membrane-anchoring fragment of the SRP receptor, and the remaining 487 amino acids (calculated M_r = 52K) comprise the cytoplasmic SRP receptor fragment. A schematic representation of the deduced amino-acid sequences of the SRP receptor and the 52K SRP receptor fragment is shown in Fig. 4A.

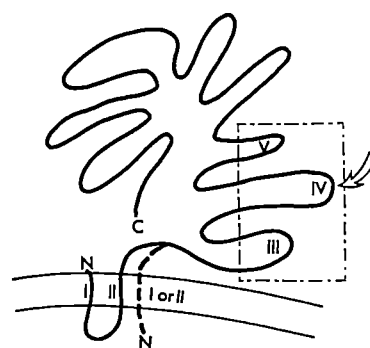


Fig. 5 Model of the disposition of the SRP receptor in the ER membrane. Regions I and/or II are the putative membrane-spanning regions; regions III–V contain the charge clusters described in the text. The arrow depicts the protease-sensitive site.

Analysis of the primary sequence for distribution of hydrophobicity reveals only two distinctly hydrophobic stretches (amino acids 1–22 and 64–79, labelled I and II in Figs 4B, 5) in the amino-terminal part of the sequence. These regions are, in principle, long enough to span the hydrophobic core of the membrane, although they are shorter than most known α -helical transmembrane segments¹⁵. However, either one or both of these regions must be in contact with the hydrophobic core of the lipid bilayer, because the intact SRP receptor can be released from the membrane only after its solubilization with detergents, and—according to the argument outlined above—it is anchored in the membrane through its amino-terminal 151 amino acids.

Apart from these two hydrophobic stretches, the remainder of the membrane-anchoring fragment of the SRP receptor is surprisingly hydrophilic. This character extends across the protease-sensitive site of the intact receptor for about 100 amino acids into the cytoplasmic fragment. In particular, the regions spanning amino acids 84–97, 129–175 and 205–243 show extreme hydrophilicity values (regions labelled III, IV and V in Figs 4B, C and 5) that are due to a remarkable abundance of charged amino acids (both basic and acidic in a relative proportion of ~2:1) which largely appear clustered (Fig. 4C). Notable is the predominantly basic character of these domains, which also dominates the overall charge of the SRP receptor (calculated pI = 9.86).

The remainder of the cytoplasmic portion of the SRP receptor contains periodically spaced regions, some of which exceed in length and hydrophobicity values those which must comprise the membrane anchor segment(s). However, as described above, on proteolysis the 52K SRP receptor fragment is readily released from the membrane, by treatment with high salt concentrations, as a rather stable and 'well-behaved' (that is, non-aggregating) soluble protein. Thus these hydrophobic stretches are probably buried within the protein. There remains the possibility that, during the functional cycle of the SRP receptor, these regions become transiently associated with the hydrophobic core of the lipid bilayer (see below).

Functional implications

When we compared the SRP receptor sequence with other known protein sequences, we found a striking resemblance of the mixed-charge amino-acid clusters (III–V in Fig. 4) to those regions in ribosomal proteins and aminoacyl-tRNA synthetases that are thought to be involved in binding of these proteins to RNA molecules^{16–19}. In addition, when the Dayhoff databank was searched for homologous protein sequences, the best match was found with sea urchin histone H1; there is 30% identity in an overlap extending from residues 120 to 201 of the SRP receptor, primarily due to the frequent occurrence of positively charged amino acids. The finding that regions III–V resemble nucleic acid-binding proteins raises the possibility that the SRP receptor could interact directly (through a domain constituted

by regions III, IV and/or V; indicated as a boxed region in Fig. 5) with the 7SL RNA in SRP, and thus become, albeit transiently, an integral part of SRP. Such a hypothesis is consistent with the fact that the SRP receptor can be eluted from an SRP affinity column (Fig. 1; ref. 9) with a relatively small increase in the magnesium concentration (from 5 to 25 mM), the ionic strength remaining constant. This small concentration difference is not likely to severely affect protein-protein interactions; however, the conformational state of RNA molecules is known to be highly dependent on the concentration of divalent cations²⁰. We therefore consider it likely that the mixed-charge amino-acid clusters III-V constitute or contribute to a domain in the molecule that is involved in its interaction with SRP. If these regions bind to the 7SL RNA molecule in SRP (as suggested by their primary structure), their separation by proteolytic cleavage (arrow in Fig. 5) could account for the apparent lack of activity of both the membrane anchoring fragment and the cytoplasmic fragment of the SRP receptor.

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Bovine opsin has more than one signal sequence

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By deletion of selected segments from a bovine opsin complementary DNA clone and subsequent analysis of transcripts in a cell-free translation-translocation system, we have localized two out of four theoretically conceivable signal sequences required for the integration of opsin into microsomal membranes.

THE *in vitro* translation of messenger RNAs for integral membrane proteins in the presence of an appropriate acceptor membrane (representing the *in vivo* site of integration) has proved to be a useful approach for studying the mechanism by which proteins are asymmetrically integrated into membranes.

Experiments of this kind were first carried out using glycoprotein G of the vesicular stomatitis virus as a model integral membrane protein and dog pancreas microsomal membranes as a model acceptor membrane¹⁻³. These experiments suggested that the initial events in the integration of the G protein into microsomal membranes were similar to those that occur in the translocation of secretory proteins. In both cases, the proteins were synthesized with transient amino-terminal signal sequences that were cleaved during or shortly after translocation³. Moreover, the signal sequences of these two proteins were similar and used the same translocation machinery in the microsomal membrane³. Translocation, as well as integration, was shown to be dependent on the signal recognition particle (SRP): translocation (integration) did not occur with KCl-washed (SRP-depleted) microsomal membranes (KRM) and the re-addition of SRP restored this ability (for summary, see ref. 4).

Several proteins that are not synthesized as larger precursors and therefore do not have cleaved signal sequences have been studied using the above assays. Two different integration

mechanisms could be distinguished experimentally⁵⁻¹⁰. In one case, exemplified by cytochrome *b₅*, integration into the lipid bilayer occurs spontaneously and independently of SRP⁵⁻⁸. The segment within the protein that specifies such spontaneous insertion into membranes has been termed an 'insertion' sequence¹¹. In the other case, exemplified by Ca²⁺-ATPase⁸, integration is specified by a signal sequence and is catalysed by SRP.

The present study used bovine opsin as a model for integral membrane proteins that are not synthesized as larger precursors and are polytopic¹¹. Opsin traverses the membrane seven times, resulting in four separate hydrophilic domains being exposed on the exoplasmic side of the microsomal membrane and four on the cytoplasmic side (for summary, see refs 12, 13). We shall call 'trans' domains those portions of the molecule that have been translocated across the membrane such that they extend into the microsomal vesicle lumen (representing the 'outside' of the cell). Those portions that are not translocated across the microsomal membrane and remain in the biosynthetic compartment of the cell are called 'cis' domains. Hypothetical considerations¹¹ indicate that several possible mechanisms could establish this topology. For example, each of the translocated domains may require a separate signal sequence. If this were the case, opsin may contain as many as four uncleaved signal sequences, one for each domain to be translocated. Alternatively, a single

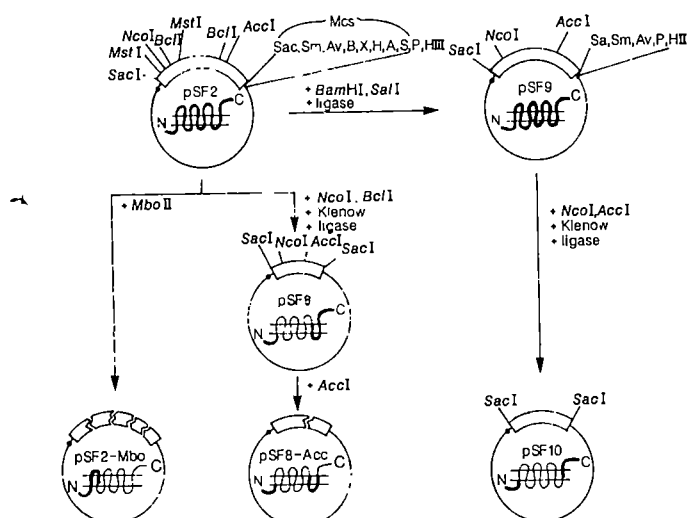


Fig. 1 Construction of opsin-containing SP6 vectors. Mcs, multiple cloning site; Sac, *SacI*; Sm, *SmaI*; Av, *AvaI*; B, *BamHI*; X, *XmaI*; H, *HincII*; A, *AccI*; S, *SalI*; P, *PstI*; HIII, *HindIII*; N, amino-terminus; C, carboxy-terminus; solid circle, SP6 polymerase binding site. A schematic representation of the various forms of opsin encoded by each plasmid is included within each large circle. The two parallel lines represent the membrane lipid bilayer and the opsin molecule is depicted by a waveform that traverses the membrane seven times. The darkened portion of each opsin represents the portion of the molecule encoded by the respective plasmids.

Methods. A cDNA clone (bd20) encoding the entire opsin apoprotein of bovine rhodopsin was obtained from Dr J. Nathans (Stanford University)¹⁴. This 1,639-nucleotide clone was removed from pBR322 by cutting it with *SacI* and ligating the resulting 1,382-base pair (bp) restriction fragment into the *SacI* site of Sp65 (ref. 17). This plasmid (pSF2) was used to transform a *dam*-methylase⁺ strain of *Escherichia coli*, GM48. The insert contained nucleotides 36–1,414 of bd20, 60 nucleotides of the 5'-untranslated region, a coding region of 1,044 nucleotides and 274 3'-untranslated nucleotides. pSF2 was used to prepare various truncation or deletion recombinants. All cloning procedures were as described by Maniatis *et al.*²⁸ and restriction digests were carried out in conditions recommended by New England Biolabs. Recombinant plasmids, as described below, were identified by restriction endonuclease digestion and used to synthesize mRNA¹⁷. Construction of specific plasmids: pSF2-Mbo. This truncated form of opsin (encoding amino acids 1–93) was obtained by digesting pSF2 with *MboII*. Multiple fragments were generated, but only one 710-bp piece contained the SP6 polymerase binding site continuous with the first 311 nucleotides of the opsin insert. pSF8. To obtain this plasmid, pSF2 was digested with *NcoI* and *BclI*. The resultant 3' recessed termini were filled-in with Klenow fragment and ligated to produce a recombinant with the correct opsin reading frame, a retained *NcoI* site and no *BclI* site. pSF8-Acc. This truncated DNA, prepared by digesting pSF8 with *AccI*, encoded a 79-residue protein. pSF9. Identical to pSF2 except that 8 nucleotides of the SP65 multiple cloning site were deleted by digesting pSF2 with *BamHI* and *SalI* and re-ligating the linearized vector. This was done to remove the *AccI* site from the multiple cloning site so that the *AccI* site at nucleotide 961 in the opsin coding region became a unique restriction site. pSF10. This plasmid encodes 83 amino acids of opsin, including the 34 amino-terminal residues continuous with, in frame, the carboxy-terminal 48 residues. An additional Cys residue at position 35 is a result of filling-in and ligating. Thus, a form of opsin is obtained which contains none of the transmembrane segments and only the first *trans* and the last *cis* domains. To obtain pSF10, pSF9 was digested with *NcoI* and *AccI*, filled-in with Klenow and ligated, resulting in the loss of the *NcoI* and *AccI* sites and the acquisition of an additional *NlaIII* site.

signal sequence may be used for the translocation of the NH₂-terminal translocated domain (which is glycosylated at two sites) followed by three insertion sequences, one each for the three remaining translocated domains. Combinations of signal and insertion sequences (one for each translocation domain) are also conceivable. Finally, it is possible that opsin does not contain signal sequences and that its integration occurs exclusively by insertion sequences.

To distinguish between these possibilities and to localize at least some of the 'topogenic' sequences required for integration¹¹, we carried out manipulation at the DNA level, either by truncating a full-length complementary DNA clone of bovine opsin¹⁴ or by cutting the cDNA clone and ligating various segments of interest. The resulting constructs (as well as the uncut full-length cDNA) were then transcribed and the RNAs

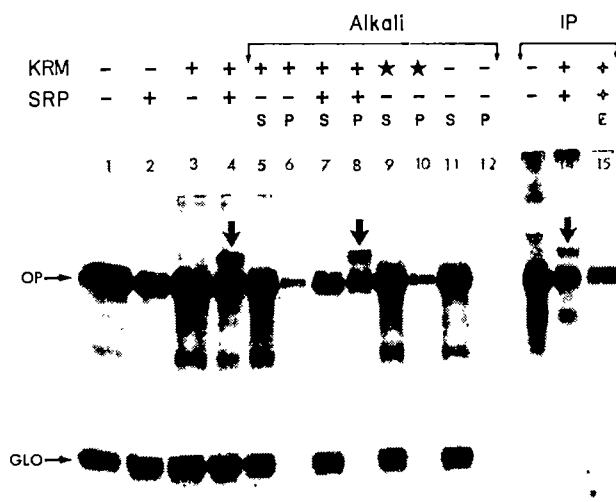


Fig. 2 Opsin is integrated into microsomal membranes in an SRP-dependent fashion. SP6-generated opsin mRNA¹⁷ and total rabbit reticulocyte RNA were translated in a wheat-germ system with (+) or without (–) SRP (800 U ml^{–1}) or salt-washed membranes (KRM, 2 µl) in a total reaction volume of 24 µl. In lanes 9 and 10, the stars indicate that KRM were present only during extraction with alkali, not during translation. Following translation, samples in lanes 5–12 were treated with alkali (pH 11.0) for 10 min on ice, centrifuged into an alkaline sucrose cushion for 10 min at 30 p.s.i. in a Beckman airfuge and then separated into supernatant (S) and pellet (P) fractions before solubilization and gel loading. The samples in lanes 13 and 14 were immunoprecipitated with antiserum to opsin and then prepared for SDS-PAGE. In lane 15, the sample was treated with Endo H (E) and then immunoprecipitated with anti-opsin serum. OP, primary translation product of opsin; GLO, α and β-globin; IP, immunoprecipitates; alkali, alkali-extracted. Downward-pointing arrows indicate the position of the glycosylated form of opsin.

Methods. Sp6-generated mRNAs were prepared from linearized plasmids (see Fig. 1) as described by Melton and co-workers¹⁷. After transcription, mRNAs were purified by phenol/chloroform extraction and ethanol and then lithium precipitation²¹. The wheat-germ *in vitro* translation system has been described previously¹⁸; 60 or 100 ng of pSF2 mRNA and 20 A₂₆₀ U of total rabbit reticulocyte RNA were added to each 24-µl reaction mixture. Translations were carried out for 40–60 min at 23 °C and stopped by chilling on ice and precipitation with an equal volume of cold 20% trichloroacetic acid. Preparation of KRM and SRP was as described previously^{29,30}; 2 µl KRM was used with 20 U SRP for each 24-µl translation mixture. Alkali extraction and alkaline sucrose gradient centrifugation were carried out according to Gilmore and Blobel²³. Digestion with Endo H was for 12 h as described previously³¹. The SDS-PAGE system has been described elsewhere¹⁶.

translated in a wheat-germ cell-free translation system in the presence or absence of SRP-depleted microsomal membrane and/or the absence or presence of SRP. Our data suggest that opsin contains at least two signal sequences.

Opsin integration requires SRP

Our first objective was to determine whether full-length opsin, which is not synthesized as a larger precursor¹⁵, contains a signal sequence or whether its integration into microsomal membranes¹⁶ is mediated entirely by insertion sequences. A construct of full-length opsin DNA in SP6 (pSF2; see Fig. 1) was transcribed¹⁷ and the RNA translated in a wheat-germ cell-free system¹⁸. Rabbit reticulocyte RNA (coding primarily for the two globin chains) was included in all translations (unless otherwise noted). The synthesized globin served as an internal

control, as its synthesis should not be affected by SRP and it should not be integrated into microsomal membranes. Opsin and the two globin chains (the latter were not resolved from one another) were indeed the major translation products (Fig. 2, lane 1). Addition of 20 units of SRP caused inhibition of opsin synthesis without affecting the synthesis of globin (Fig. 2, lane 2). These data suggested that opsin contains a signal sequence which, by analogy to the cleaved NH₂-terminal signal sequences of secretory proteins¹⁹, caused an SRP-mediated elongation arrest. A discrete arrested fragment (or several discrete fragments if there are several signal sequences) has so far not been detected.

More definitive evidence for the existence of a signal sequence in opsin was the synthesis of a glycosylated form of opsin in the presence of SRP and KRM (SRP-depleted membranes) (Fig. 2, lane 4, downward-pointing arrow). As expected, this glycosylated form was sensitive to treatment with endoglycosidase H (Endo H) (Fig. 2, compare lanes 14 and 15). The synthesis of a glycosylated form of opsin indicated that at least the NH₂-terminal domain of opsin was translocated. Note that the glycosylated form of opsin was synthesized only when both KRM and SRP were present. In the absence of SRP only the non-glycosylated form was synthesized (Fig. 2, lane 3). The SRP-mediated inhibition of opsin synthesis was largely abolished in the presence of KRM (Fig. 2, compare lane 2 with lanes 3, 4). These data are consistent with release of an SRP-mediated elongation arrest by KRM¹⁹.

Although the SRP-dependent appearance of a glycosylated form of opsin suggested a signal sequence-mediated mechanism of translocation (at least for the glycosylated NH₂-terminal domain of opsin), it was conceivable that the three *trans* domains further downstream were integrated by insertion sequences (for example, independently of SRP). In this case, the non-glycosylated opsin synthesized in the presence of KRM only (Fig. 2, lane 3) might also be integrated. As a criterion for integration, we used non-extractability at alkaline pH (pH 11)^{8,20}. When synthesized in the absence of SRP, the non-glycosylated form of opsin was not integrated into KRM, as >90% was extracted by alkali (Fig. 2, compare lanes 5 and 6). The small amount of opsin that was resistant to alkali extraction was not integrated into KRM, but, rather, represented aggregated material co-sedimenting with KRM. This aggregated material was observed even when KRM and NaOH were added together (Fig. 2, lanes 9, 10) or when KRM were omitted altogether (Fig. 2, lanes 11, 12). Thus, it appears that insertion sequences are not used for the integration of opsin. However, these data alone do not exclude the possibility that translocation of the NH₂-terminal domain of opsin by a signal sequence is required for the subsequent expression of putative insertion sequences that may serve to integrate the other three translocated domains.

As expected, the glycosylated form of opsin synthesized in the presence of SRP was not extracted at alkaline pH (Fig. 2, compare lanes 7 and 8). In addition, when synthesized in the presence of SRP, a significant amount of the non-glycosylated form of opsin was resistant to alkali extraction. This non-glycosylated, non-extractable form of opsin probably represented opsin that had been integrated but not glycosylated. This could be a result of an inefficiency of dog pancreas microsomal membranes to glycosylate relative to their ability to translocate. Alternatively, if opsin possesses separate signal sequences for each translocated domain, the first one (responsible for translocation of the first *trans* (glycosylated) domain) might have been missed, yielding non-glycosylated opsin integrated via any or all of the other signal sequences. Note that all of the globin chains were completely extracted by alkali in any of the experimental conditions used (Fig. 2, lanes 5–12) and thus behaved as predicted for a non-integrated, cytoplasmic protein. Although resistance to alkali extraction does not prove that glycosylated opsin was integrated correctly (that is, with all four *trans* domains properly translocated), we can conclude that this was the case for at least the first *trans* domain, containing the two glycosylation sites.

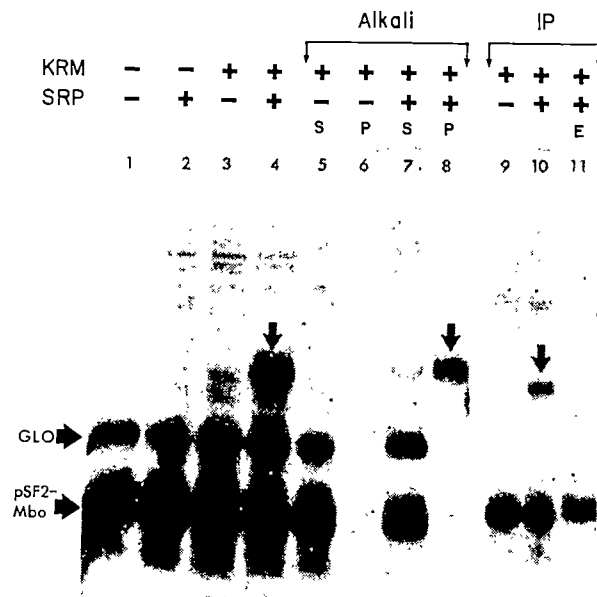


Fig. 3 The first 93 amino-terminal residues of opsin contain a signal sequence. pSF1 was digested with *Mbo*II and the resultant truncated plasmid (pSF2-Mbo) was used to generate mRNA translated in the wheat-germ system with (+) or without (-) SRP or KRM as described in Fig. 2 legend. The samples in lanes 5–8 were treated with alkali as described in Fig. 2 legend. The translation products in lanes 9–11 were immunoprecipitated with anti-opsin serum and those in lane 11 were treated with Endo H and then immunoprecipitated as described in Fig. 2 legend. Due to overexposure of this autoradiograph, the inhibition of synthesis of pSF2-Mbo is not readily apparent. When the globin and pSF2-Mbo bands were cut and quantitated¹⁹, the globin band in lane 1 contained 162,000 c.p.m. and that in lane 2 contained 171,000 c.p.m.; for pSF2-Mbo, these counts were 236,000 (lane 1) and 138,000 (lane 2). After correcting the pSF2-Mbo values for sample variability¹⁹, it was calculated that pSF2-Mbo synthesis was inhibited by 63% in the presence of SRP.

Localization of opsin signal sequence

Our initial strategy to localize the signal sequence was to determine the minimum length of opsin necessary for integration. The opsin-containing SP6 plasmid was cut with various restriction enzymes to generate a number of truncated opsins consisting of progressively shorter NH₂-terminal ends of the molecule. The shortest truncated form, pSF2-Mbo, encoded the 93 NH₂-terminal amino acids of opsin (calculated relative molecular mass (*M*_r) 12,300), and consisted of the first *trans* domain, the first transmembrane segment, the first *cis* domain and most of the second transmembrane segment. Translation yielded a product of the expected mass (Fig. 3, lane 1). Translation was inhibited in the presence of SRP (Fig. 3, compare lanes 1 and 2). Moreover, a glycosylated form of the 93-residue translation product was synthesized only in the presence of both KRM and SRP (Fig. 3, lane 4, downward-pointing arrow), not with KRM alone (Fig. 3, lane 3). As expected, the glycosylated form was not extracted by alkali (Fig. 3, compare lanes 7 and 8), whereas the non-glycosylated form synthesized in the absence of SRP was extracted (Fig. 3, compare lanes 5 and 6). The non-glycosylated and glycosylated forms of this polypeptide could be immunoprecipitated with an antiserum against a synthetic peptide representing residues 14–23 of opsin (Fig. 3, lanes 9–11). Furthermore, the glycosylated form was sensitive to Endo H digestion (Fig. 3, lane 11).

These data suggest that a signal sequence is localized in the first 93 residues of opsin. Of these, 30–40 carboxy-terminal residues can be expected to be sequestered in the ribosome^{21,22}. As the signal sequence is presumably expressed only when exposed on the surface of the ribosome¹⁹, the 30–40 carboxy-terminal residues of the 93-amino-acid polypeptide (which

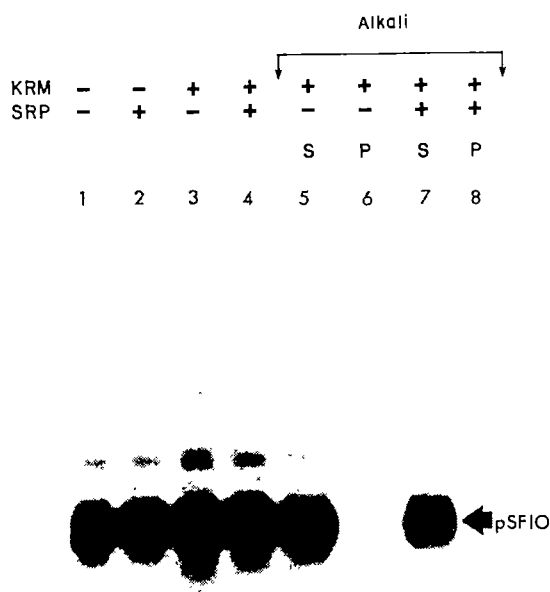


Fig. 4 The amino-terminal *trans* domain of opsin does not function as a signal sequence. A form of opsin containing only the amino-terminal 34 residues fused to the carboxy-terminal 37 residues (encoded by pSF10) was analysed for SRP-dependent integration and translocation. Reticulocyte RNA was omitted from the translation mixture because the proteins encoded by pSF10 and globin have similar electrophoretic mobilities (data not shown). pSF9-10 (300 ng) was used to programme the wheat-germ translation system in the presence (+) or absence (-) of KRM and SRP as described in Fig. 2 legend. The samples in lanes 5-8 were treated with alkali as described for Fig. 2.

include the second transmembrane segment and much of the first *cis* domain) could therefore not have served as a signal sequence. Thus, the first 53-63 residues (comprising the first *trans* domain and the first transmembrane segment) must contain the potential sites for a signal sequence.

To determine which of these two domains might contain a signal sequence, we constructed a plasmid (pSF10) in which most of the first *trans* domain was ligated to the fourth *cis* domain (the latter is highly charged and is not expected to contain a signal sequence). Since the translation product of this construct (calculated M_r 10,630) co-migrated with the globin chains (data not shown), reticulocyte RNA was omitted from the translation. The data shown in Fig. 4 indicate that this construct does not contain a signal sequence: there was no significant SRP-mediated inhibition of translation (Fig. 4, compare lanes 1 and 2), no SRP-mediated appearance of a glycosylated form (Fig. 4, compare lanes 3 and 4), and no SRP-mediated alkali-resistant integration (Fig. 4, compare lanes 5-8). We therefore conclude that a signal sequence is not localized in the region of the glycosylated *trans* domain, but, rather, in the region comprising the first transmembrane segment.

A second signal sequence

As discussed above, opsin could contain as many as four signal sequences, one for each domain to be translocated. To localize a second signal sequence, we ligated the DNA encoding the first *trans* domain to that encoding a carboxy-terminal portion of opsin beginning with the sixth transmembrane segment (Fig. 1). The transcribed RNA of this construct (pSF8) yielded a translation product with a predicted M_r of 16,500. It is clear that this opsin construct contains a signal sequence (Fig. 5a). Its translation was inhibited by SRP. Moreover, the synthesis of a glycosylated form (Fig. 5a, lane 4, downward-pointing arrow) that resisted alkali extraction was dependent on the presence of SRP (Fig. 5a, compare lanes 1-4). As neither the amino-terminal *trans* domain nor the carboxy-terminal *cis* domain contains a signal sequence (see Fig. 4), the signal

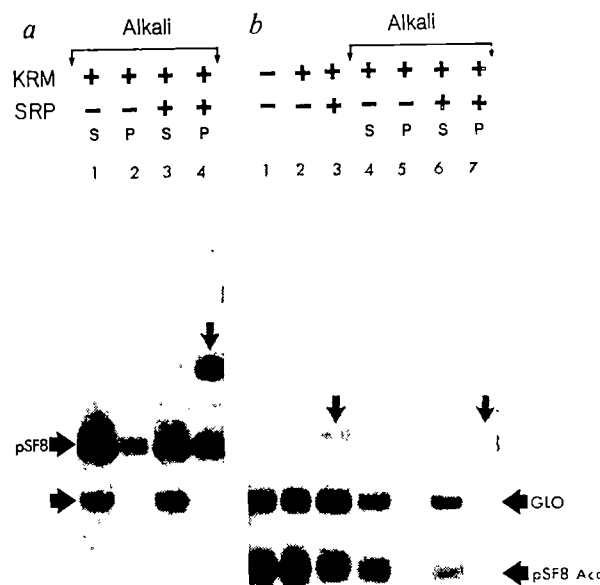


Fig. 5 A second signal sequence is located in the sixth transmembrane segment. *a*, An opsin construct (pSF8) containing the first and fourth *trans* domains, transmembrane segments VI and VII and the fourth *cis* domain, was analysed for SRP-dependent integration and translocation. pSF8 (150 ng) was used to programme the wheat-germ translation system in the presence (+) or absence (-) of KRM or SRP as described in Fig. 2 legend. Following translation, the samples were alkali-treated as described for Fig. 2. *b*, A truncated form of pSF8 (pSF8-Acc) (encoding the first *trans* domain, sixth transmembrane segment, *trans* loop 4 and 14 residues of the seventh transmembrane segment) was used to programme the wheat-germ translation system and the translation product was analysed for SRP-dependent integration and translocation as described for *a*. Following translation, the samples displayed in lanes 4-7 were extracted with alkali as described for Fig. 2. The glycosylated forms (downward-pointing arrows) were Endo H-sensitive (data not shown).

sequence of this construct must be localized in a region comprising transmembrane segments 6 and 7.

To localize the signal sequence more precisely, we prepared a truncated form of this construct (pSF8-Acc), resulting in the removal of the fourth *cis* domain and part of the seventh transmembrane segment. Of this translation product, 30-40 of the carboxy-terminal residues would not be exposed on the ribosome and therefore could not function as a signal sequence. Thus, if this construct contains a signal sequence, it must be localized in the sixth transmembrane segment, specifically in the amino-terminal portion of this segment. Figure 5b shows that a signal sequence was, in fact, present in this construct. A glycosylated form (Fig. 5b, lanes 3, 7, downward-pointing arrows) was synthesized only in the presence of SRP and was resistant to alkali extraction (Fig. 5b, compare lanes 4-7). We emphasize that although these data show the presence of a signal sequence in the amino-terminal portion of the sixth transmembrane segment, our experiments do not rule out the possibility that the seventh transmembrane segment also has the potential to function as a signal sequence.

In the two cases (Figs 3, 5b) where we have used truncated forms of opsin (lacking a termination codon), we have analysed the translation mixture by sucrose gradient centrifugation and subsequent SDS-polyacrylamide gel electrophoresis (PAGE) of gradient fractions. Surprisingly, in both cases ~60% of the truncated chains were released from the ribosomes (data not

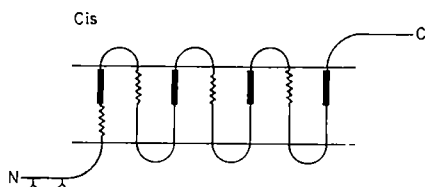


Fig. 6 Model illustrating the possible locations of signal and stop transfer sequences within opsin. See text for explanation. N, amino-terminus; C, carboxy-terminus; Cis, cytoplasmic side of membrane; zigzag lines, signal sequences; thickened portion of line, stop transfer sequences; λ , glycosylation sites.

shown). As a result, the 30–40 carboxy-terminal residues of these released chains were no longer located within the ribosome and, thus, a signal sequence located within this region could potentially interact with the membrane. However, the recent demonstration²³ that signal sequences addressed to the endoplasmic reticulum (ER) can be expressed only when presented to the ER in the context of the ribosome (that is, only when the chain is attached to the ribosome) rules out the possibility that the observed integration was due to the expression of a signal sequence exposed after chain release.

Perspectives

The most important conclusion from our data is that the integration of a polytopic integral membrane protein with multiple translocated domains may require multiple signal sequences. From hindsight, opsin turned out to be a fortunate choice. Its first *trans* domain of 36 residues contains the only two glycosylation sites (at residues 2 and 15) of the protein. We showed that this domain does not function as a signal sequence. In the deletion experiments described above, the first *trans* domain could always be retained and therefore be used as a convenient 'reporter' domain in the search for signal sequences elsewhere in the molecule; the readily detectable glycosylation of this domain in the *in vitro* translation-translocation system indicated that translocation of this domain had taken place. By definition, whenever this translocation event was SRP-dependent, a signal sequence mediated the event. So far we have localized two signal sequences, one in the first transmembrane segment, the other in the amino-terminal portion of the sixth transmembrane segment. We assume that in full-length opsin the first of these would function in the translocation of the first (glycosylated) *trans* domain and the latter in the translocation of the fourth *trans* domain. Signal sequences are also likely to be used for translocating the second and third *trans* domains and might be located in the amino-terminal portions of transmembrane segments 2 and 4 (Fig. 6).

Among the important unresolved questions is the precise topology of the various constructs in the microsomal membrane. It is likely, however, that the first NH_2 -terminal *trans* domain, when glycosylated, is localized on the *trans* side of the membrane, since asparagine-linked glycosylation requires translocation²⁴.

Another unresolved question is how the signal sequence-induced translocation process (which goes to completion in the case of secretory proteins) is interrupted in the case of membrane proteins. One model proposes that a translocation is halted by a 'stop transfer' sequence which in turn triggers disassembly of a chain-conducting proteinaceous channel in the membrane¹¹. Alternative models propose that translocation is arrested by the interaction of hydrophobic stretches of the polypeptide chain with the lipid bilayer while the chain is passing across the bilayer (that is, the chain does not pass through a proteinaceous channel^{25,26}).

Figure 6 shows a scheme for the possible location of alternating signal and stop transfer sequences in opsin. The dynamics of the integration process could be envisaged within the framework of a previously proposed model¹¹. The interaction

of the first signal sequence with the membrane would proceed as described recently for secretory proteins (for summary, see ref. 4): the signal sequence, in the form of a loop^{26,27}, is recognized by SRP and a translation arrest is induced; the translation arrest is released by an interaction of the SRP with its cognate receptor (SRP receptor) in the ER, resulting in the displacement of SRP from the ribosome. These interactions bring the translating ribosome into the vicinity of the ER and would thus facilitate the subsequent interaction of the signal sequence with a putative signal receptor (which could be represented by signal peptidase) and of the ribosome with a putative ribosome receptor, both integral membrane proteins of the ER. The amino-terminal *trans* domain would then traverse the membrane through the proteinaceous channel (which might be formed, at least in part, by the signal receptor and the ribosome receptor). The signal sequence would be displaced from its ER receptor following further chain elongation. Passage of the chain would eventually be interrupted by the appearance in the channel of a stop transfer sequence. As a result, the channel would open to the lipid bilayer and the first integrated transmembrane segment would, after lateral displacement, be entirely surrounded by lipid molecules. Further chain elongation would result in the extra-ribosomal exposure of the second signal sequence (again in the form of a loop) which would initiate a second round of translocation. A second stop transfer sequence would stop the translocation again. Two more rounds of translocation-initiation and translocation-termination would result in the proper topology for opsin. It is conceivable that SRP and its cognate receptor in the ER are involved only in targeting the first signal sequence to the membrane. The second, third and fourth signal sequences, in a shortened circuit, may be able to interact directly with the signal receptor in the membrane without first interacting with SRP. Although it is unknown whether such a shortened circuit (which bypasses recognition of the signal sequence by SRP and uses only the putative signal receptor in the ER) could occur *in vivo*, integration *in vitro* mediated by the fourth signal sequence (even though tested here separately and out of context) requires SRP.

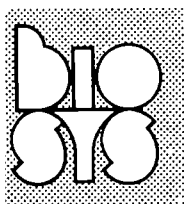
Many of the predictions illustrated in our model (Fig. 6) can be tested. Thus, the two additional predicted signal sequences could be localized by experiments similar to those described here. Similarly, it may be possible to separate the signal sequences from the putative stop transfer sequences by designing constructs that retain one of the signal sequences, but none of the stop transfer sequences. Such constructs would be expected to be completely translocated.

The approaches used here may serve as a paradigm for determining which topogenic sequences (insertion sequences, signal sequences) are used for the integration of polytopic integral membrane proteins and precisely where within the protein these sequences are located.

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LETTERS TO NATURE

Radio emission from the jet and lobe of 3C273

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The 'superluminal' motion observed in the cores of radio sources such as 3C273 is now accepted^{1,2} as evidence of relativistic motion within a few parsecs of the centre, but it is less clear whether such speeds persist out to kiloparsec scales^{3,4}. The one-sidedness of such sources is often cited as evidence of relativistic Doppler beaming, but could equally be intrinsic. New maps of 3C273 at 151 and 408 MHz have been made using the MERLIN interferometer⁵, with dynamic range of $4 \times 10^3:1$ and $10^4:1$, respectively. These show that (1) there is an extended region or lobe to the south of the main jet, (2) the radio emission of the jet is continuous from the core to beyond the limit of the optical jet and (3) no counter-component can be found in the opposite direction to the jet. The ridge-line of the jet shows a 'wobble', the wavelength (λ) of which decreases by a factor of 6 along its length. We suggest a model in which the jet motion is relativistic throughout, the velocity decreasing from $\beta\gamma \approx 6$ to $\beta\gamma \approx 1$ (where β is the velocity and γ the Lorentz factor). Since the Doppler beaming factors calculated for this model are too small to hide the lobe or the head of a counter-component, we suggest that 3C273 is intrinsically one-sided.

The MERLIN interferometer⁵ was equipped for operation at 151 MHz in early 1985, and observations were made of 3C273 at this frequency on 1985 May 21. The resulting 151-MHz map at 3.0 arc s resolution is shown in Fig. 1 (the first MERLIN map to be published at this frequency), while Fig. 2 shows a 408-MHz map at 1.0 arc s resolution which was obtained by processing earlier observations⁶ using a new software procedure called MAP (R. G. Noble, A. Bailey and T.W.B.M., unpublished). This procedure, which uses the hybrid mapping method⁷, corrects both telescope errors and closure offsets in amplitude and phase. Furthermore, we have removed the effects of the confusing double source⁶, situated 8 arcmin away from 3C273. These techniques result in a considerable improvement in dynamic range. A 408-MHz map was also made at 3 arc s resolution (not shown) for comparison with Fig. 1.

The previously known features of the radio source^{3,6,8} are the flat-spectrum core coincident with the quasar, which is unresolved at 1 arc s resolution, and the steep-spectrum radio jet extending 22 arc s in position angle 222.5°, leading to a bright head. (The head contains three sub-components⁹ which are merged at 1 arc s resolution.)

An entirely new feature in Figs 1 and 2 is an extended low-brightness region on the southern side of the jet. By analogy with classical double radio sources, we refer to this feature as the 'lobe'. There is no trace of extended emission on the northern side to the limit of measurement. In Fig. 2 the lobe has a well-defined outline about halfway along the jet, but there is additional extended emission close to the side of the jet out to

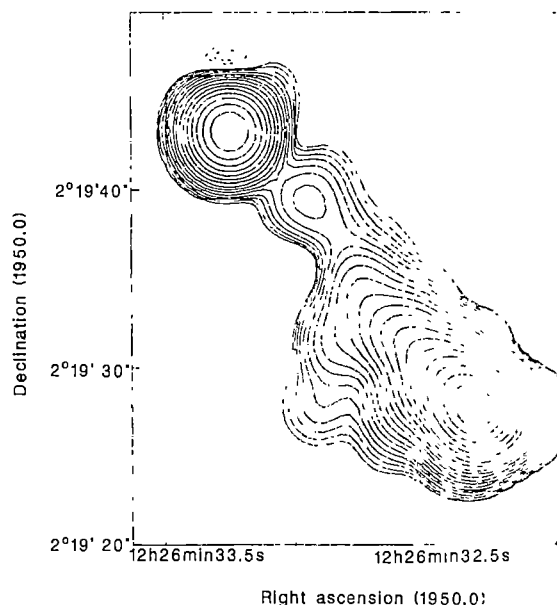


Fig. 1 MERLIN map of 3C273 at 151 MHz with 3.0 arc s resolution. Peak brightness is 55.8 Jy per beam. Contours are at equal logarithmic intervals, two contours to a factor 2, above a bottom contour of 56 mJy per beam. Negative (dashed) contours are at -56 and -80 mJy per beam.

20 arc s, appearing to spread backwards from the head at an angle of about 20°. At the lower resolution of Fig. 1, the 151-MHz lobe emission is blended with that of the main ridge, but is more intense and more extended than at 408 MHz. The spectral index α_{151}^{408} (obtained by comparing the two maps at 3 arc s resolution) is 1.5 ± 0.3 in the lobe and 0.80 ± 0.05 in the jet. From consideration of the integrated spectrum, Foley and Davis⁶ showed that the low-frequency radiation must come from a hitherto undetected area some 50 (arc s)² in area. This value matches the area of the outermost contour in Fig. 1, and we suggest that the lobe accounts for most of the flux at frequencies < 20 MHz¹⁰.

The centre of the lobe is near the inner end of the optical jet, but there is no measured optical radiation from the lobe. (There is a blue extension on the north side¹¹, where there is no radio emission; this may be merely a coincidence.) The X-ray emission reported¹² as coming from the jet of 3C273 is also located near the inner end of the jet, and could arise in the radio lobe itself.

Both the morphology and the spectral index suggest that the lobe consists either of remnant material left behind after the passage of the active head, or of backflow. In either case, any motion will be slow, and the lobe emission will not be relativistically beamed. We have calculated physical parameters of the relativistic plasma in the lobe using standard formulae¹³, with $H_0 = 100 \text{ km s}^{-1} \text{ Mpc}^{-1}$, so that 1 arc s = 1.85 kpc. We assume the line-of-sight thickness to be 5 kpc. If as much kinetic energy is assigned to the positive ions as to the electrons in the plasma, the minimum pressure in the lobe is $10^{-10} \text{ dyn cm}^{-2}$, the equipartition magnetic field is $\approx 60 \mu\text{G}$, and the synchrotron lifetime at 151 MHz is $5 \times 10^6 \text{ yr}$. The lobe must be at least this

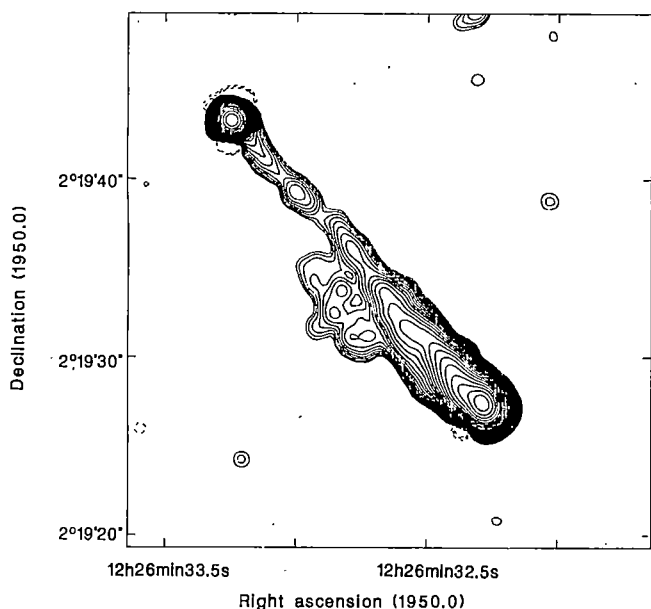


Fig. 2 MERLIN map of 3C273 at 407 MHz with 1.0 arc s resolution. Peak brightness is 13.5 Jy per beam. Contours are at equal logarithmic intervals, two contours to a factor 2, above a bottom contour of 6.75 mJy per beam. Negative (dashed) contours are at -6.7 and -9.5 mJy per beam. Positions in both Figs 1 and 2 have been calibrated by adjusting the position of the core to right ascension 12 h 26 min 33.248 s; declination +02° 19' 43.29" (VLA Calibrator list).

old if the steep spectral index indicates ageing. During this time, the head has moved forward some 10 arc s or 20 kpc, implying that the forward motion of the head is sub-relativistic, with a transverse component of velocity of the order of 0.01 c .

It might initially be supposed that the southward displacement of the lobe was caused by a transverse ambient 'wind' in the cluster of which 3C273 is a member¹⁴. However, the difficulty with this interpretation is that the ram pressure ρv^2 for the wind (where ρ is the ambient density and v the wind velocity) should be at least equal to the pressure in the lobe. If the wind velocity is of the order of 300 km s⁻¹, the required density corresponds to 10^{-1} H atoms cm⁻³. Such a high density is hard to reconcile with the radio polarization data¹⁵: the rotation measure of all parts of the source is ≤ 1.5 rad m⁻², and hence the integral $\int n_e B dl$ in front of the source is $\leq 10^{-6}$ cm⁻³ G pc (where n_e is the electron density, B is the magnetic field and l is the path length). There is thus an unresolved discrepancy, unless the ambient intergalactic gas is singularly devoid of magnetic field ($B < 10^{-10}$ G over 100 kpc).

In all previous pictures, both radio and optical, the core and jet have been separated by a gap of 10 arc s. The new maps show that the radio emission of the jet continues across the gap as a faint narrow ridge, unresolved in width (angle $\theta \ll 1$ arc s), and with brightness $\approx 10^{-3}$ of the peak. In the brighter part of the jet, the width of the ridge apparently increases, but this may be solely due to blending with the lobe. The ridge can be traced over the whole 21 arc s of length, and Fig. 3a shows the intensity profile at 408 MHz along the ridge-line, plotted logarithmically. The rise to the peak follows an exponential increase with an e-folding scale height of ≈ 2 arc s, or 4 kpc. Similar profiles are found at 151 MHz (Fig. 1) and also at 1.67 GHz⁶ and 5 GHz⁸, with little or no change in scale height. If this region were a trail, subject to synchrotron ageing as in the lobe, the scale height would vary as $\nu^{0.5}$. We suggest instead that the intensity profile indicates the rate at which energy is extracted from bulk kinetic energy and dumped into the synchrotron reservoir.

In Fig. 3b the position of the ridge-line is plotted relative to an axis through the core at position angle 222.5°. Close to the head, the pattern can be followed best at 1.67 GHz, and the last five points are taken from the $\lambda 18$ cm map at 0.35 arc s resolution⁶. The 'wobble' in the ridge-line^{3,6} is present throughout the

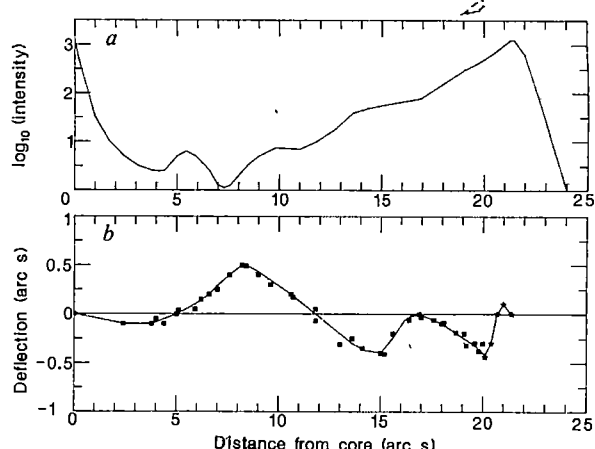


Fig. 3 a, Plot of intensity (log scale) along the ridge of the jet; b, deflection of ridge-line from a straight line through the core in position angle 222.5°. (The vertical scale is exaggerated $\times 5$.) ■, 408 MHz; *, 1,666 MHz.

length of the jet, superimposed on a centre-line which curves gently southwards by $\approx 2^\circ$ in position angle. The amplitude of the wiggle is constant, at about 0.5 arc s peak to peak, but the semi-wavelength of the pattern, measured between crossing-points, decreases systematically from 6 arc s to ≈ 1 arc s.

Wiggles are observed in many sources and have not been satisfactorily explained. However, the most straightforward interpretation of the systematic decrease in wavelength is as a deceleration of the jet material. If we interpret the wiggle in terms of precession in the central object with period T , the pattern wavelength λ is proportional to T and to the apparent transverse velocity β_{obs} :

$$\lambda = cT\beta_{\text{obs}} = cT\beta \sin \theta / (1 - \beta \cos \theta)$$

On this interpretation, the decrease of pattern wavelength down the jet indicates that deceleration begins at 10 arc s from the core, and continues to the head. The rate of deceleration thus mimics the rise in radio intensity. Because little braking is apparent in the inner part, we propose that the initial value of β_{obs} equals the superluminal value observed a few marc s from the core. The proper motion of 0.79 marc s yr⁻¹ (ref. 16) means that, if $H_0 = 100$ km s⁻¹ Mpc⁻¹, $\beta_{\text{obs}} = 4.8$ and T is 15×10^3 yr. Figure 3b then suggests that β_{obs} decreases to a value ≈ 0.8 at the point where the jet enters the head.

The degree of polarization observed at $\lambda 18$ cm in the brightest sub-component of the head⁹ has been explained in terms of relativistic aberration. If the Doppler blueshift factor is

$$\eta = (\gamma(1 - \beta \cos \theta))^{-1}$$

the aberration needed to account for the polarization (see ref. 9) requires that

$$\eta \sin \theta = \sin 47^\circ$$

By combining this value with the value of β_{obs} from the pattern wavelength, we may deduce the speed β and the angle θ of the motion near the head. If the value of θ does not change, parameters may also be obtained for the motion near the core.

Table 1 Model parameters for the motion in the jet reckoned in the frame of the observer

Region	Near core	Near head
Distance from core	≤ 10 arc s	21 arc s
Speed, β	0.987	0.74
Angle, θ	19°	19°
Lorentz, γ	6.2	1.5
Doppler blueshift, η	2.3	2.2
Apparent transverse velocity, β_{obs}	4.8	0.79
Factor $x = (1 + \beta \cos \theta) / (1 - \beta \cos \theta)$	28	5.5

Table 1 shows values for these quantities at each end of the jet, all values being expressed in the observer's frame of reference. The Lorentz factor of the initial velocity, when reckoned in the rest-frame of the quasar ($z=0.158$), is $\gamma \approx 7$.

The velocity model presented here is consistent with the radio data, but is not unique. In particular, the parameters could vary widely in value if the jet were bent, that is, if θ varied along the length. We note that a bend of position angle occurs at 8 marc s from the core¹⁶, and that most of the measurements of superluminal motion refer to distances less than 8 marc s. A crucial test of our model would be provided by measurement beyond the bend. According to the model, the proper motion should remain at $0.79 \text{ marc s yr}^{-1}$ at all points out to 10 arc s, decreasing to $<0.1 \text{ marc s yr}^{-1}$ near the head. Further measurements of the proper motion at high dynamic range and long wavelengths would help to resolve this question.

The observations presented here may be used to test whether or not the one-sidedness of 3C273 is due to Doppler beaming. The observed peak brightness in the head at 408 MHz is 13 Jy per beam at 1 arc s resolution, and 22 Jy per beam at 3 arc s. Identifiable sidelobes due to residual errors are present at the 30-dB level (1,000:1 down) north and south of bright features. Away from these points the r.m.s. noise level is 1.2 mJy per beam at 1 arc s resolution, or 1.9 mJy per beam at 3 arc s (these values are the observed r.m.s. values over several hundred square arc s in empty regions of the map, and are $\approx 50\%$ above the calculated thermal noise). Thus, the observed dynamic range at 408 MHz (defined as peak intensity per r.m.s. level) is $10^4:1$. At 151 MHz the corresponding values are 56 Jy per beam peak brightness, r.m.s. level 13 mJy per beam, giving a dynamic range of $4 \times 10^3:1$. Along the continuation of the major axis from the quasar out to the geometrical counter-head position 22 arc s away the 408-MHz map shows no emission above noise level, with a maximum value of 4 mJy per beam (2σ) near the geometrical counter-head.

We have calculated the intrinsic front/back ratio in luminosity for each component of the source, allowing for the Doppler beaming factors of any that are fast-moving. At the head the observed brightness at 3 arc s resolution is 22 Jy per beam, so that the observed head/counter-head ratio is $>5,500:1$. The expected brightness ratio for two equal jets in opposite directions is $x^{2+\alpha}$, where $x = (1 + \beta \cos \theta)/(1 - \beta \cos \theta)$. For the model suggested above with $\beta = 0.74$ and $\theta = 19^\circ$, the Doppler beaming ratio is 120. Even if the entire radiation from the head were beamed with this Doppler factor, the expected intensity from a counter-jet would be 190 mJy per beam, making the front/back ratio for the jet $\geq 47:1$. In practice the head almost certainly emits a mixture of beamed and unbeamed radiation. Flatters and Conway⁹ suggested that 1/6th of the head flux comes from a slow-moving precursor component with little beaming. The intrinsic front/back ratio on this basis is $\geq 1,000:1$.

The lobe emits at least 500 mJy unbeamed radiation at 408 MHz from an area of $15 (\text{arc s})^2$, while no region of equal area on the opposite side of the core emits more than 10 mJy. Thus, the observed front/back ratio for the lobe is $\geq 50:1$, in sharp distinction from classical double sources. While the high front/back ratios for the head are model-dependent, being based on our assumptions of jet speed, the front/back ratio for the lobe is free from such assumptions.

We conclude that there is no observational evidence of any counter-components in 3C273. Doppler beaming is insufficient to hide the counterpart of the lobe and probably that of the head. If such counterparts do exist, they must be intrinsically weaker than those observed by one or two orders of magnitude. Thus, 3C273 contains all the ingredients of a classical double source like Cygnus A, with core, jet, hotspots and extended lobe, but only on one side.

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Implications of ultra-high-energy emission from Hercules X-1

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A 3-min outburst of very-high energy (VHE) quanta, $E > 10^{12} \text{ eV}$, was recently detected from the X-ray pulsar Hercules X-1 (ref. 1). The outburst occurred at a time during the 35-day X-ray modulation that is associated with X-ray turn-on. Temporal analysis of the outburst showed the same 1.24-s modulation that is observed at X-ray energies. Subsequent monitoring of the system at ultra-high energies (UHE) by the Fly's Eye² yielded evidence for a 40-min outburst of quanta with energies $E > 1 \times 10^{14} \text{ TeV}$. This UHE outburst also exhibited a 1.24-s modulation. Here we assume the quanta to be γ -rays and show how the interaction of these UHE particles with a precessing accretion disk can explain the observed γ -ray 'light' curve. We also discuss the possibility that the emitting UHE particles are accelerated by shocks in an accretion flow as well as the constraints that can be placed on the acceleration mechanism.

Hercules X-1 displays X-ray modulation with periods of 1.24 s, ~ 1.7 days and ~ 35 days^{3–5}. The simplest interpretation of the two shorter periodicities is that they are due to rotation and occultation of an accreting neutron star located in a close binary system^{3–7}. The unusual 35-day flux modulation has an X-ray light curve that is composed of an 11-day high-intensity state and a 19-day low-intensity state, interrupted midway between the 11-day high states by an intermediate high state (intensity $\sim 40\%$ of main high state) of 5 days duration^{3,4}. This modulation is usually referred to by the varying aspect of an inclined accretion disk that precesses about the X-ray pulsar^{5–7}. In this picture, the 19-day low state occurs when our view of the pulsar is obscured by the accretion disk and the 11-day high state occurs when our view is unobstructed. The intermediate high state occurs when our line-of-sight passes close to the disk and is partially obscured by the disk's corona.

An intriguing aspect of the high-energy observations is the phase at which the γ -ray outbursts occur during the 35-day X-ray modulation. The UHE outburst occurred on 11 July 1983 at phase $\phi_{35} = 0.63$ in the 35-day cycle². This epoch corresponds to the middle of the period, when the intermediate high state is normally observed. The VHE outburst occurred on 17 April 1983¹, the nominal time for the onset of the 11-day high-intensity state. Both outbursts occurred at a time when the accretion disk is situated so that our line-of-sight was grazing the disk.

An attractive explanation for these observations is provided by a particular variation in the class of beam dump models⁸ in which the accretion disk serves as the beam dump. It is related to the model we later proposed⁹⁻¹¹ for the reported post-eclipse emission of Cygnus X-3, which has also been applied to LMCX-4 (ref. 12). The model for Her X-1 assumes that UHE particles are accelerated in a co-rotating region near the pulsar and then stream outward to interact with the surrounding accretion disk. Energetic γ -rays will then be detectable when the beam of particles crosses our line-of-sight and interacts with a column thickness of material that is comparable to the particle's radiation length. A smaller thickness would be inefficient as a converter, whereas a larger column thickness would obscure the photons. In the Her X-1 system, this condition is usually best met at the onset and decline of the X-ray high states when our line-of-sight is grazing the precessing accretion disk. In fact, the VHE outburst did occur at the nominal time for the onset of the 11-day X-ray high state. However, the production of the UHE outburst during a phase normally associated with the centre of the secondary X-ray high state would require a thickening of the disk to yield sufficient target along our line-of-sight. The Exosat X-ray satellite observations of the source during the same period support this idea¹³. The satellite's failure to detect the X-ray high states, despite the continuing optical variability attributed to the reprocessing of X rays, led several authors^{13,14} to conclude independently that the disk was significantly thicker during this epoch.

The duty cycle of the UHE emission is only about 10% of the spin period², which we take to imply beaming of the UHE primaries.

We next consider the efficiency of particle acceleration in the Her X-1 system. The median energy of 500 TeV quoted by the Fly's Eye group² is based on the assumption that the γ rays have a power law spectrum. The apparatus in fact responded to γ rays $>1 \times 10^{14}$ eV (Elbert, J. W., personal communication). Taking, as a conservative estimate, the photon energies to be $E = 1 \times 10^{14}$ eV, a reported time-averaged UHE photon flux of 3×10^{-12} cm⁻² s⁻¹ (ref. 2) and a duty cycle of 0.1, we derive a peak UHE energy flux of 3×10^3 eV cm⁻². This should be compared with the total X-ray flux of 4×10^3 eV cm⁻². The conversion efficiency for UHE particles to photons is only $\sim 10\%$. A liberal estimate for the beaming factor can be made by assuming that the broadest part of the beam passes through our line-of-sight. The solid angle of the beam is then $\pi^3 \times 10^{-2}$, and the beaming factor is $\pi^2/4 \times 10^{-2}$. We conclude that the UHE particle luminosity is at least $\sim 25\%$ of the X-ray luminosity.

This sets strong constraints on models of particle acceleration. For example, acceleration scenarios¹⁵ that invoke the rotational energy of the accretion disk encounter the difficulty of that for Her X-1 parameters, which include a surface magnetic field of 4×10^{12} G, the inner radius of the accretion disk is located at more than 300 stellar radii. It is difficult to see how more than about 1/300th of the total energy budget could be channelled through rotation of the accretion disk. The fact that the UHE and VHE emission has the periodicity of the neutron star's rotation further supports the notion that the energy that goes into the UHE particles is liberated close enough to the neutron star for the in-falling material to co-rotate with it.

The high efficiency associated with shock acceleration makes it an attractive possibility for particle acceleration in the Her X-1 system. In particular, one expects a shock from the impact of the accreting material onto the star or its magnetosphere. Given that spontaneous injection occurs¹⁶, shock acceleration can take place¹⁷. It is not necessary for the shock to occur at the surface of the neutron star for the mechanism to put much of the released gravitational energy into UHE particles; all that is needed is that much of the pressure in the post-shock material that settles on the surface is in the form of UHE particles trapped in the flow. However, our understanding of shock acceleration enables us to place strong constraints on models that invoke it.

The synchrotron loss timescale for a particle of mass m , energy

$E_p = \gamma mc^2$ and charge Ze is given by

$$\tau_{sy}(E_p) = \frac{4\pi mc}{\gamma \sigma_T B^2} \left(\frac{m}{Z^2 m_e} \right)^2 \quad (1)$$

where B is the magnetic field strength in the region and σ_T is the Thompson cross-section. The time required to accelerate a relativistic particle to energy E_p is

$$\tau_a(E_p) = (\xi R_g) / (\beta_s c) \quad (2)$$

where R_g is the gyroradius ($R_g = \gamma mc^2 / (ZeB)$), ξR_g is the mean free path ($\xi \geq 1$) and $\beta_s c$ is the shock velocity. The maximum energy E_d to which a particle can be accelerated by a shock, even in the absence of synchrotron losses, is given by¹⁸

$$E_d = \frac{1}{\pi} \beta_s Z e B R \quad (3)$$

where R is the radius of the shock, which must be less than the size of the region. This limit comes from the fact that particles with energies greater than E_d can diffuse away from the system within the acceleration timescale. Stipulating that the acceleration must be faster than the synchrotron loss, and combining this limit with equation (3), we find that the maximum energy $\gamma_m mc^2$ to which a particle can be accelerated within a compact region of size R is given by

$$\gamma_m = \beta_s \left[\left(\frac{3}{2\pi\xi} \right) \frac{Rm}{Z^2 r_0 m_e} \right]^{1/3} \quad (4)$$

where r_0 is the classical electron radius.

If the shock velocity is taken to be the free fall velocity at a radius R from the neutron star, $V_{ff} = (2GM_*/R)^{1/2}$, then individual particle energies as high as $9 \times 10^6 mc^2 R_6^{-1/6}$ are possible (where $R = R_6 \times 10^6$ cm). This is sufficient to account for the UHE emission from Her X-1. Note also that this upper limit is rather pessimistic because it uses a classical equation for the synchrotron loss timescale. In fact, this loss rate is an overestimate when radiation reaction and quantum mechanical effects become important.

A final constraint, we note, is that the Alfvén Mach number of the shock must be high if much of the energy is to go into the highest-energy particles. That is

$$\rho_s u_s^2 \gg \frac{B^2}{8\pi} \quad (5)$$

where ρ_s is the preshock fluid density and u_s is $\beta_s c$. If ρ_s is fixed by the condition that

$$\rho_s u_s (\pi R^2) = \dot{M} = \frac{L}{(\epsilon c^2)} \quad (6)$$

where \dot{M} is the accretion rate, and is the conversion efficiency from accreted mass to luminosity L , this constrains the magnetic field to be less than $\beta_s^{1/2} L_8^{1/2} R_6^{-1/2} \times 10^8$ G, and R to be less than 10^8 cm. The synchrotron loss limit also constrains B to be less than $7 \times 10^{21} \xi \gamma_m^{-2}$ G. We see then that the model demands that the magnetic field within the accretion column be much less than the surface field of the neutron star. This is reasonable because the accreting material is a good conductor and is likely to pass between field lines of the neutron star on its way to the surface. However, it is not clear to what extent the flow retains its coherence between the magnetopause and the stellar surface. Alternatively, one could invoke a shock at 10-50 neutron star radii, where the field strength of the star's magnetosphere is 10^7 - 10^9 G.

We have proposed that the UHE emission reported from Her X-1 is generated by particles that interact with the surrounding accretion disk. The model predicts that high-energy γ -ray outbursts should occur preferentially at the onset and decline of the high-intensity X-ray states. The acceleration of the primaries occurs within the neutron star's magnetosphere, and the most obvious mechanism is acceleration by the accretion shock. A substantial fraction of the γ -rays may be generated within the

accretion column, the relative contribution of these two targets depending on the γ - γ opacity. Accretion-shock acceleration can account for the highest observed γ -ray energies, but not by a wide margin, and would be ruled out by detection of photons at 10^{16} eV or higher. Photons at $E > 10^{16}$ eV have been claimed by several authors, implying primary energies of $\sim 10^{17}$ eV (refs 12, 19). If these energy measurements are correct to within a factor of two, and if deep inelastic scattering behaves at these high energies as at lower ones, then according to our analysis, acceleration by accretion shocks cannot account for the primary energies. We emphasize the importance of precise energy measurements; γ -ray energies of $2\text{--}3 \times 10^{15}$ eV are marginally consistent with accretion-shock acceleration of the primaries. On the other hand, a shock created by a relativistic wind striking, say, an accretion disk at $R > 10^8$ cm, could generate primary energies of 10^{17} eV. Relativistic winds could result from supercritical accretion. It might be useful therefore, to search for correlations between E_{max} and evidence for relativistic winds.

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Relationship between peroxyacetyl nitrate and nitrogen oxides in the clean troposphere

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Nitrogen oxides have a key role in the chemistry of the polluted and the unpolluted atmosphere^{1–4}. It has been proposed that important quantities of nitrogen oxides may be contained in the form of peroxyacetyl nitrate (PAN) especially in the colder regions of the middle and upper troposphere^{5,6}. Surface and aircraft observations of PAN have confirmed its ubiquity in the remote troposphere where it has been measured at mixing ratios of $10\text{--}500$ p.p.t.v. (parts per 10^{12} v/v) (refs 7, 8). To date, no study that is representative of the remote atmosphere has been made to assess directly the concentrations of PAN compared with that of the inorganic nitrogen oxides (NO and NO₂). Here we present the first study in which the mixing ratios of PAN, and nitrogen oxides—as well

as those of peroxypropionyl nitrate (PPN) and O₃ and relevant meteorological parameters—were measured concurrently at a location that receives clean, continental air. The results show that in clean conditions nitrogen oxides present in the form of PAN can be as much or more abundant than the inorganic form.

The measurements were made at the C-1 research site of the Mountain Research Station of the University of Colorado (40°2' N lat., 105°32' W long., 3.05-km elevation above sea level). Prevailing winds at this site are from the west and are relatively free of anthropogenic pollution. However, there are frequent occasions when winds from the east transport anthropogenic pollution to the site. The measurements, then, are representative of very clean to polluted conditions and must be interpreted in the context of the prevailing meteorology. This field study was conducted during the summer (1–16 July 1984; 24 August–2 September 1984) and the autumn (24 October–4 November 1984). The autumn period resembled early winter in that snow cover, high winds and low temperatures were typical.

The instruments used to measure NO_x, PAN and PPN, and O₃ have been described elsewhere^{9–11}. Briefly, NO was measured with a chemiluminescent technique and NO₂ was photolysed to NO before detection. The instrument has detection limits of 2 p.p.t. for NO and 10 p.p.t. for NO₂ based on a 10-s averaging time. The absolute accuracy of these measurements is estimated to be 15% for NO, 30% for NO₂ and 40% for NO_x. Unlike many instruments that catalytically reduce NO₂ to NO, the interference of PAN in the present NO₂ photolysis system was found to be <5% in laboratory tests^{9,10}. PAN and PPN were measured with two different instruments, both employing electron capture gas chromatography and cryogenic sample collection¹. Although the two PAN instruments did not operate concurrently, an intercomparison of results during similar atmospheric conditions indicated no systematic differences. With a 100 cm^3 (STP) sample the PAN, PPN instruments had a detection limit of ~ 5 p.p.t., a precision of $\pm 10\%$ and an estimated accuracy of 20–30%. O₃ was measured by a UV-absorption instrument (Dasibi, Inc., Model 1003-AH) with a detection limit of 3 p.p.b.v. (parts per 10^9 v/v) and an estimated uncertainty of $\pm 10\%$.

The relationship between the mixing ratios of PAN and NO_x are examined in Fig. 1. Summer and autumn data from two periods of the diurnal cycle are presented. First is an afternoon period (1100 to 1800 MST) that is characteristic of the results of the day's photochemical activity. Second is the photochemically- and meteorologically-quiescent night-time period (000 to 800 MST) of late night to early morning. In the summer afternoon, [PAN] increases with increasing [NO_x]. This behaviour is expected as PAN is rapidly produced by photochemistry in summer, at least in polluted air masses, and [NO_x] is a good indicator of the degree of urban pollution at this site⁹. In contrast, in the autumn and in the summer night-time the abundance of PAN is nearly constant and independent of the NO_x mixing ratio.

Figure 2 shows the mean diurnal behaviour of [PAN]. The symbols show the average PAN mixing ratio for periods when the NO_x mixing ratio was 300 p.p.t.v. or less. This [NO_x] criterion generally coincides with westerly winds at the site and is chosen as representative of relatively clean continental air masses. Figure 2 indicates that relatively constant PAN levels are encountered at this site in the summer and in the autumn in clean atmospheric conditions. The absence of a distinct diurnal cycle in the PAN levels indicates that PAN production and loss are approximately in balance throughout the day in clean continental air in the boundary layer troposphere. For these clean air masses the daily average PAN level was higher in the autumn (243 p.p.t.v.) than in the summer (170 p.p.t.v.). The PPN data gave results similar to those for PAN during both seasons at all NO_x levels with an average [PPN]/[PAN] ratio of 0.05.

For comparison, the mean diurnal behaviour of all the [PAN] data for these two seasons is also presented in Fig. 2 and is shown as the dashed lines. Here, the PAN mixing ratio does show a strong afternoon maximum in the summer but not in

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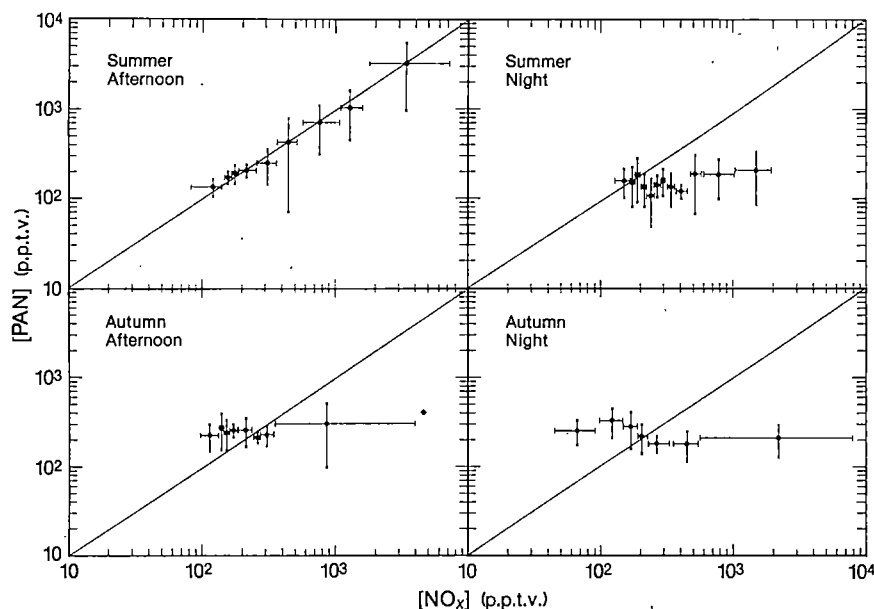


Fig. 1 PAN mixing ratio as a function of the NO_x mixing ratio. For both the summer and autumn sampling periods, afternoon (1100–1800 MST) and night-time (000–800 MST) data are given. Each set of data was arranged in order of increasing $[\text{NO}_x]$. Each symbol gives the $[\text{PAN}]$ and $[\text{NO}_x]$ averages of 10 consecutive $[\text{NO}_x]$ values. The vertical bars indicate the standard deviation ($\pm 1\sigma$) of the $[\text{PAN}]$ values and the horizontal bars show the range of NO_x mixing ratios included in each average. The diagonal lines indicate equal mixing ratios for PAN and NO_x .

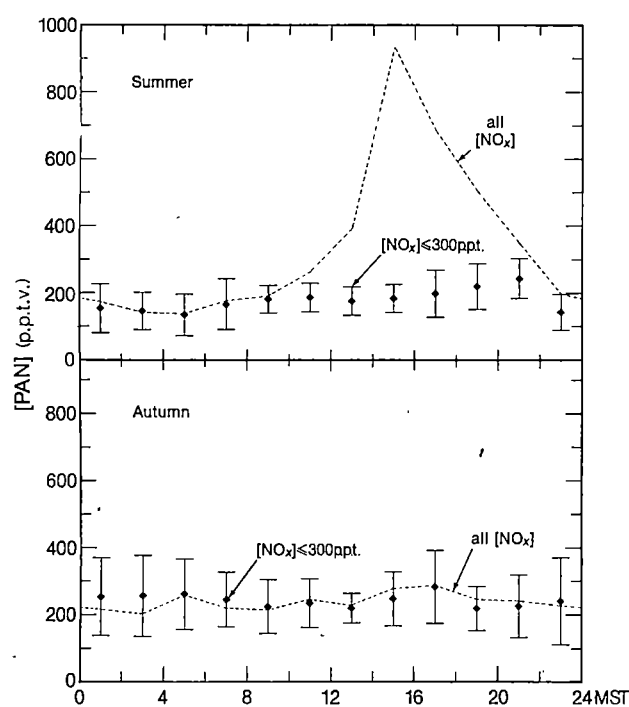


Fig. 2 Diurnal variation of PAN mixing ratio in the summer and in the autumn. The symbols represent averages of $[\text{PAN}]$ for a 2-h period of the diurnal cycle for the subset of the total measurements that is defined by $[\text{NO}_x] \leq 300$ p.p.t.v. The vertical bars indicate the standard deviation ($\pm 1\sigma$) of the $[\text{PAN}]$ values. The dashed lines given the corresponding averages for the full data set (all $[\text{NO}_x]$ values).

the autumn. This summer peak is attributed to both photochemistry and meteorology. During the summer, along the Front Range in the Colorado Mountains, the easterly, upslope winds increase in frequency during the daytime as a result of the daytime heating of the mountains. These easterly winds bring air containing recent (≤ 12 h) additions of pollution from the Denver metropolitan area, including high concentrations of both PAN and NO_x . The summer afternoon PAN peak observed at this site is similar in shape to that observed by Lewis *et al.*¹² in the summer in New Jersey, but with lower absolute values.

The importance of PAN relative to NO_x is shown in Fig. 3. The $[\text{PAN}]/[\text{NO}_x]$ ratio increases with decreasing $[\text{NO}_x]$ values below 500 p.p.t.v. in both summer and autumn. This increase, particularly in the autumn, is principally due to the

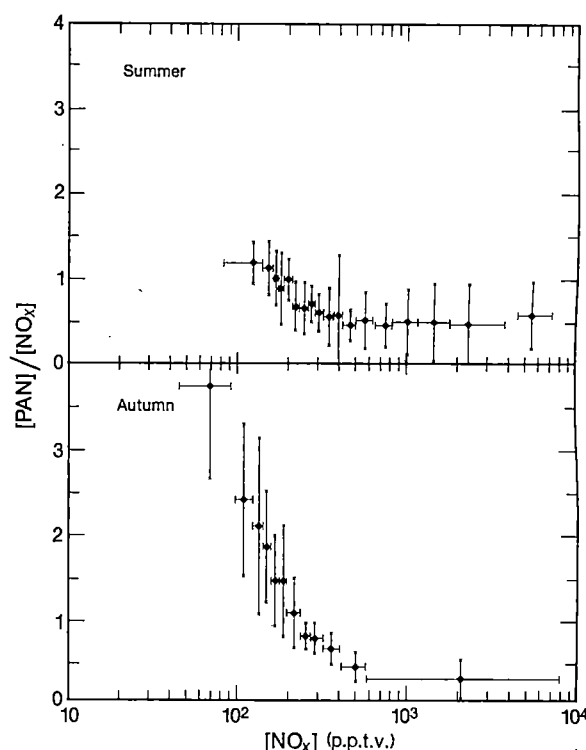


Fig. 3 $[\text{PAN}]/[\text{NO}_x]$ ratio as a function of $[\text{NO}_x]$ for the summer and the autumn. Each data set was arranged in order of increasing $[\text{NO}_x]$. Each symbol gives the $[\text{PAN}]/[\text{NO}_x]$ and $[\text{NO}_x]$ averages of 20 consecutive $[\text{NO}_x]$ values. The vertical bars indicate the standard deviation ($\pm 1\sigma$) of the ratio values while the horizontal bars show the range of $[\text{NO}_x]$ included in each average.

PAN levels remaining constant as $[\text{NO}_x]$ decreases. For NO_x levels < 300 p.p.t.v., the average $[\text{PAN}]/[\text{NO}_x]$ ratios are 0.5 to ≥ 1 in summer and 0.5 to ≥ 3 in autumn. The higher $[\text{PAN}]/[\text{NO}_x]$ ratio in autumn can be attributed, at least in part, to the increased stability of PAN at the lower autumn temperatures. No other determinations of the $[\text{PAN}]/[\text{NO}_x]$ ratio at comparable NO_x mixing ratios have been reported. However, there are other determinations at higher NO_x levels^{13,14} which are consistent with our results.

Figure 4 shows the observed relationship between the PAN and O_3 mixing ratios. High PAN levels are associated with high O_3 concentrations in the summer but the two are essentially decoupled in the autumn. This relationship between O_3 and PAN suggests that both PAN and O_3 are produced photochemi-

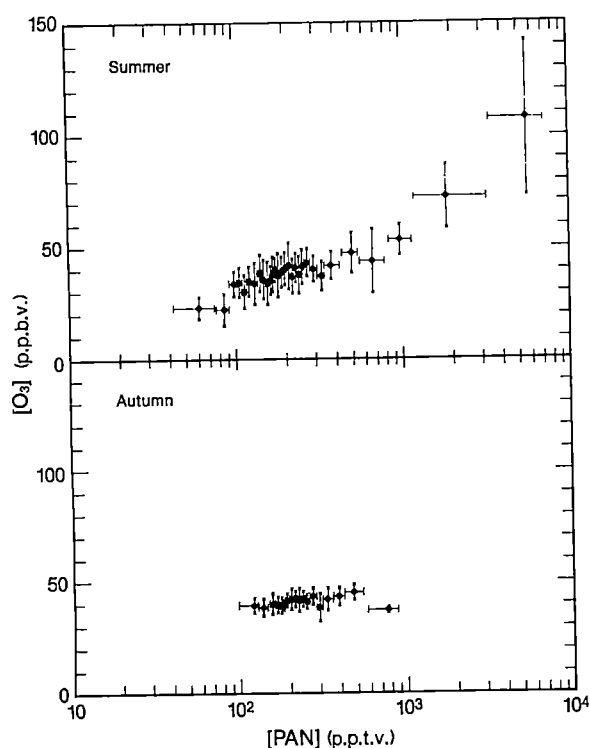


Fig. 4 Ozone mixing ratio as a function of [PAN] for the summer and the autumn. Each data set was arranged in order of increasing [PAN]. Each symbol then gives the [O₃] and [PAN] averages of 15 consecutive [PAN] values. The vertical bars indicate the standard deviation ($\pm 1\sigma$) of the [O₃] values while the horizontal bars show the range of [PAN] included in each average.

cally near the site. The photochemical production of O₃ at this site has been observed and reported earlier^{15,16}. The independence of the PAN, O₃ and NO_x concentrations in the autumn is attributed to slower photochemistry.

The precursors of PAN are peroxyacetyl (CH₃C(O)OO, or PA) radicals and NO₂. The PA radicals are produced by the reaction of acetaldehyde and hydroxyl radicals, by the photolysis of acetone and by the reactions of methylvinyl ketone and methacrolein. Each one of these compounds is itself a secondary product of hydrocarbon oxidation. Of these, acetaldehyde is likely to be the most important PA precursor. The photochemical sink of PAN is through thermal dissociation followed by reaction of PA with NO (ref. 5). During the summer day these reactions are rapid and the PAN mixing ratio tends to follow the mixing ratio of NO₂. At night, without the presence of appreciable OH and NO, the photochemical sources and sinks of PAN are interrupted, and the PAN concentration is controlled by surface deposition and atmospheric mixing. During the autumn day, the OH levels are sufficiently reduced that PAN production is effectively suppressed while the lower temperature reduces the thermal decomposition of PAN. Consequently, the PAN levels during the autumn day are relatively independent of NO_x.

It is possible to draw more quantitative conclusions from the summer data. The abundance of acetaldehyde and PA radicals that are needed to explain the observed [PAN] values can be estimated by assuming photochemical equilibrium for PA and PAN. For the summer afternoon with relatively clean air an 'apparent' acetaldehyde mixing ratio of 150–300 p.p.t.v. and PA radical concentrations of $2\text{--}3 \times 10^7$ molecules cm⁻³ are consistent with our observations. Note that the 'apparent' acetaldehyde mixing ratio deduced is an upper limit for the actual acetaldehyde mixing ratio because PA radicals can also be formed by other paths. No measurements of acetaldehyde from remote sites are available. However, in urban/suburban environments, acetaldehyde mixing ratios in the range of 0.2–20 p.p.b.v. (refs 17–19) have been reported.

From these concurrent measurements of PAN, PPN, NO_x, O₃ and meteorological parameters at a remote site in the

Colorado mountains we conclude that nitrogen oxides present in the form of PAN can be as much or more abundant than the inorganic form. The importance of PAN relative to NO_x increases with decreasing values of NO_x as well as with decreasing temperatures. It is suggested that only a modest amount of acetaldehyde and other precursors is needed to account for this PAN synthesis. The photo-oxidation of non-methane hydrocarbons probably provides these precursors in the background air. Extrapolation of these results to the upper troposphere, for which no simultaneous PAN–NO_x data are currently available, implies a highly significant role for PAN and other organic nitrates in the transport of combined nitrogen through the atmosphere. In addition, PAN can be an important source of peroxyacetyl radicals which may be important to oxidation processes in the gas as well as liquid phases.

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Isotopic composition of atmospheric O₂ in ice linked with deglaciation and global primary productivity

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In photosynthesis, O₂ is continuously formed from H₂O and released to the atmosphere. Coupled with respiration, photosynthesis forms a loop in which oxygen isotopes are exchanged between O₂ and H₂O. During the ice ages, sea water was enriched in $\delta^{18}\text{O}$ by $\sim 1.3\%$ relative to the present value¹. Continental waters in the areas of high primary productivity exchange rapidly with the oceans. They probably presented a similar isotopic enrichment. Since the $\delta^{18}\text{O}$ of glacial water was greater than at present, we would expect that the $\delta^{18}\text{O}$ of atmospheric O₂ was also greater than at present. Fireman and Norris² and Horibe *et al.*³ have measured the $\delta^{18}\text{O}$ of O₂ from the glacial atmosphere by analysing trapped gases in ice cores. However, their data are either too few or too imprecise to demonstrate whether $\delta^{18}\text{O}$ of atmospheric O₂

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has, in fact, varied. Here we present data on the changes, during the past 22 kyr approximately, in the $\delta^{18}\text{O}$ of atmospheric O_2 trapped in the ice core Dome C (East Antarctica, 74°S , 124°E). The results show that the isotopic composition of atmospheric O_2 has indeed varied along with that of sea water, and that the $\delta^{18}\text{O}$ (O_2) record offers a tool for studying several important aspects of the global cycles of O_2 and H_2O in relation to the climate.

When the snow deposited at the surface of ice sheets transforms into ice, atmospheric air is trapped in the form of bubbles. The analysis of this fossil air extracted from ice cores has already provided spectacular information, especially on the CO_2 composition of the atmosphere during the past 40 kyr (refs 4, 5). The reliability of the measurements of the composition of trapped air depends on the absence of melting during the formation and diagenesis of the solid H_2O particles, and the lack of alteration of air bubble composition during and after sampling⁶. Based on the reliable record of atmospheric CO_2 measured on the Dome C ice core⁴, we expect that it is one of the best existing cores for determining with confidence the $\delta^{18}\text{O}$ of atmospheric oxygen during the past 25 kyr. Furthermore, the special interest of this core is due to the fact that it contains a reliable high-latitude climate record, which is reflected in the $\delta^{18}\text{O}$ of the ice⁷.

To determine the $\delta^{18}\text{O}$ of O_2 , ice samples weighing $\sim 60\text{ g}$ were placed in glass flasks and evacuated to $1\text{ }\mu\text{atm}$ pressure. Trapped air was extracted from ice by melting and slowly refreezing the water, using an experimental procedure similar to the melting method described in ref. 8. The O_2 in the extracted air was converted to CO_2 by exposure for 40 min to a hot graphite rod⁹. At the end of the combustion, water was separated from CO_2 using a frozen isopropyl alcohol trap, and the water vapour pressure was measured. The $\delta^{18}\text{O}$ of the CO_2 was determined using a Micromass 602D mass spectrometer. We found that ^{18}O fractionation during combustion is geometry-dependent and not, in general, as large as values measured by Kroopnick¹⁰. The efficiencies of air extraction and O_2 conversion to CO_2 (checked by measuring the O_2/N_2 ratio in the residual gas after combustion) were estimated to be >99 and $>99.85\%$, respectively. We did not measure residual levels of CO , which may have been formed during O_2 conversion and caused our poor reproducibility for some samples. Excluded from the compilation of our data here and in Table 1 are results from 14 samples and air standards for which the water vapour pressure after combustion was $>10\text{ }\mu\text{atm}$, the carbon rod was heated to an

anomalously high temperature, or the combustion was terminated before the standard 40-min interval. Results from nine of the excluded samples are in good agreement with data presented here. The $\delta^{18}\text{O}$ of modern atmospheric O_2 , sampled in Gif and determined using the same procedure as for ice samples, is $+23.81 \pm 0.31\%$ ($n=9$ air samples) where $\delta^{18}\text{O} = 10^3 \times ([^{18}\text{O}/^{16}\text{O}]_{\text{sample}} - [^{18}\text{O}/^{16}\text{O}]_{\text{standard}}) / [^{18}\text{O}/^{16}\text{O}]_{\text{standard}}$, the standard being standard mean ocean water (SMOW)¹¹. This value of $23.81 \pm 0.31\%$ is in satisfactory agreement with the present atmospheric value of $+23.5\%$ determined by Kroopnick and Craig¹². Our measurements on three postglacial ice samples from Antarctic ice core D10 have a mean $\delta^{18}\text{O}(\text{O}_2) = 23.68 \pm 0.26\%$, and similar values were measured for postglacial Dome C samples (Table 1). These results suggest that our extraction procedure does not fractionate isotopically the oxygen initially trapped in the air bubbles of the ice and, furthermore, that this O_2 is an isotopically unfractionated sample of the contemporaneous atmosphere. Measured $\delta^{18}\text{O}$ values of past atmospheric O_2 are given in Table 1 and Fig. 1c.

Data on the $\delta^{18}\text{O}$ of the ice in ice cores, along with that of benthic foraminifera from deep-sea sediments, provide a context for interpreting the $\delta^{18}\text{O}$ of palaeoatmospheric O_2 . Normalized $\delta^{18}\text{O}$ values of benthic foraminifera in the Indian Ocean core MD73025 (ref. 13) are shown as a function of age in Fig. 1b. The 1.6% decrease in $\delta^{18}\text{O}$ between 16 and 6 kyr BP mainly reflects the addition to the ocean of glacial meltwater depleted in ^{18}O (ref. 1), although a fraction of the signal, $\sim 0.3\%$, is probably due to a warming of the deep Antarctic circumpolar waters¹⁴. The $\delta^{18}\text{O}$ decrease associated solely with continental ice melting is believed to be $1.3 \pm 0.1\%$ (ref. 14). The $\delta^{18}\text{O}$ of ice in the Dome C ice core is shown as a function of age in Fig. 1a. The sharp increase, going towards the present, reflects the marked warming at high latitudes associated with the last glacial termination. The timescale for the ice has been obtained using a simple ice flow model⁷, assuming a variable accumulation rate related to temperature changes, as suggested by comparison with well-dated events inferred from the foraminiferal $\delta^{18}\text{O}$ profile of core MD73025 (ref. 13).

$\delta^{18}\text{O}$ of O_2 gas in the Dome C core is plotted in Fig. 1c. At a given depth gases in ice are younger than ice itself because of the time required for snow to be transformed into ice and seal the bubbles⁶. According to Schwander and Stauffer¹⁵, 80% of the bubbles close off between the snow densities 0.795 and 0.830. The mean ages of the gas have been calculated assuming

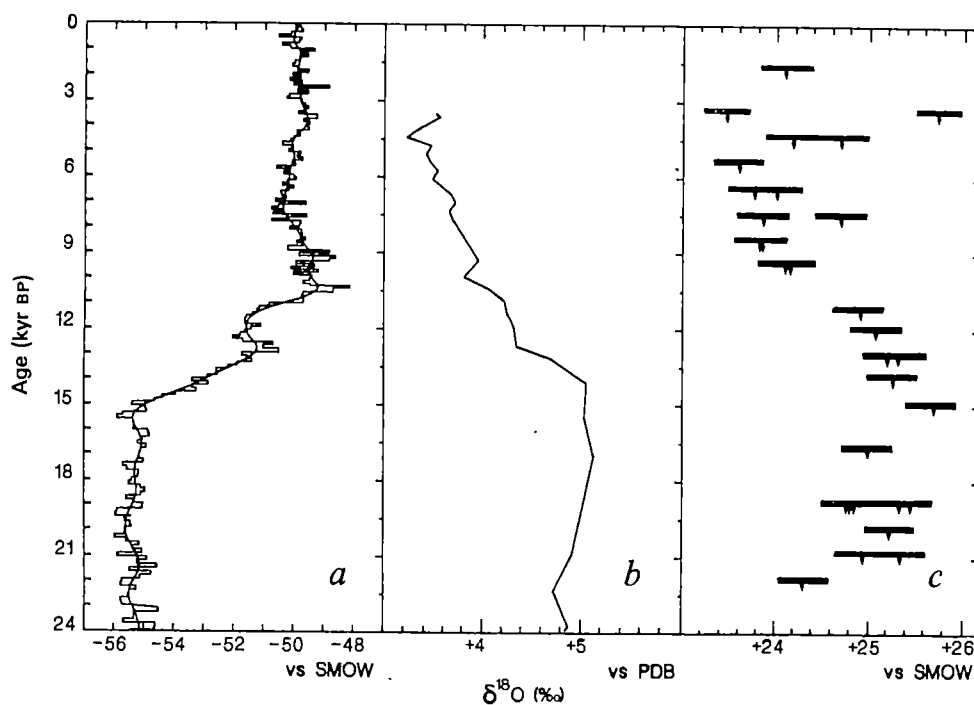


Fig. 1 a, $\delta^{18}\text{O}$ of ice versus age of the ice. This plot is identical to that in ref. 7 except that the ordinate here is linear with age. b, $\delta^{18}\text{O}$ of benthic foraminifera in core MD73025 versus age. c, $\delta^{18}\text{O}$ of O_2 gas in air trapped in Dome C versus mean age of the gas. The horizontal bars correspond to the analytical uncertainty of $\pm 0.25\%$, and the arrows indicate that the mean age of the gas may be older by a few hundred years.

Table 1 $\delta^{18}\text{O}$ of O_2 in air trapped at different depths in Dome C, along with ice equivalent depth, age of the ice and estimated mean age of the gas at each level

Depth below the surface (m)	Depth in ice equivalent (m)	Age of the ice (kyr)	Mean age of the gas (kyr)	$\delta^{18}\text{O}$ of the O_2 (% vs SMOW)
150.9	118.6	3.26	1.56	24.04
210.8	179.1	4.97	3.27	25.61
210.8	179.1	4.97	3.27	23.45
246.2	215.1	6.00	4.30	24.10
246.2	215.1	6.00	4.30	24.62
279.2	248.1	6.96	5.26	23.57
315.6	283.5	8.03	6.33	23.70
315.6	283.5	8.03	6.33	23.97
350.2	319.1	9.06	7.36	24.62
350.2	319.1	9.06	7.36	23.82
384.3	353.2	10.08	8.38	23.78
384.3	353.2	10.08	8.38	23.80
414.4	383.3	10.98	9.27	24.05
414.4	383.3	10.98	9.27	24.09
476.9	445.8	13.03	11.09	24.81
500.8	469.7	13.91	11.86	24.97
530.4	499.3	15.07	12.87	25.21
530.4	499.3	15.07	12.87	25.12
552.3	521.2	15.99	13.73	25.14
578.3	547.2	17.10	14.84	25.54
578.3	547.2	17.10	14.84	25.02
618.1	587.1	18.82	16.56	24.89
667.5	636.5	20.99	18.73	24.68
667.5	636.5	20.99	18.73	25.17
667.5	636.5	20.99	18.73	25.31
667.5	636.5	20.99	18.73	24.74
667.5	636.5	20.99	18.73	24.77
690.7	659.7	22.02	19.76	25.13
712.7	681.6	23.01	20.75	24.83
712.7	681.6	23.01	20.75	25.23
736.4	705.3	24.08	21.81	24.24

that the bubbles close off at a constant rate between the densities 0.8 and 0.83. The corresponding mean age values are given in Table 1. However, different factors discussed in ref. 16 may accelerate the close off, and the mean ages of the gas may, therefore, be older by a few hundred years in the case of Dome C. This effect is suggested by the arrows in Fig. 1. Two important results emerge from the $\delta^{18}\text{O}(\text{O}_2)/\text{age}$ curve (Fig. 1c). First, during the last glacial period, the mean $\delta^{18}\text{O}$ of atmospheric O_2 was higher than the present value by 1.3%. Second, the change in the $\delta^{18}\text{O}$ of atmospheric O_2 and that of sea water (inferred from foraminiferal $\delta^{18}\text{O}$) occurred approximately simultaneously, given the uncertainties in dating procedures and scatter in the data.

Concerning the first point, the average $\delta^{18}\text{O}$ of samples younger than 7 kyr is $+23.76 \pm 0.25\%$ ($n = 5$) whereas the average $\delta^{18}\text{O}$ for samples older than 15 kyr is $+25.06 \pm 0.24\%$ ($n = 14$). Anomalous results on samples from depths of 211 and 736 m have been excluded in this averaging. Their inclusion increases the standard deviation but does not significantly change the mean values (giving $+23.90\%$ for samples younger than 7 kyr, and $+25.00\%$ for samples older than 15 kyr). Our results thus confirm the expectation discussed earlier, that the $\delta^{18}\text{O}$ of atmospheric O_2 was heavier during the last glacial than it is today. We believe that we can dismiss the possibility that this glacial/postglacial $\delta^{18}\text{O}(\text{O}_2)$ difference is due to oxygen isotope exchange in the ice. First, no inorganic reactions between H_2O and O_2 are known which occur in the absence of light at low temperatures. Second, with an ice $\delta^{18}\text{O}$ around -50% and a fractionation factor at equilibrium of $\sim 20\%$ (ref. 17), the $\delta^{18}\text{O}$ of trapped O_2 approaching isotopic equilibrium with ice would decrease progressively during ageing (to about -30%). The opposite effect is observed.

The change in the $\delta^{18}\text{O}$ of atmospheric O_2 between the last glacial and the present (1.3%) is identical to the corresponding

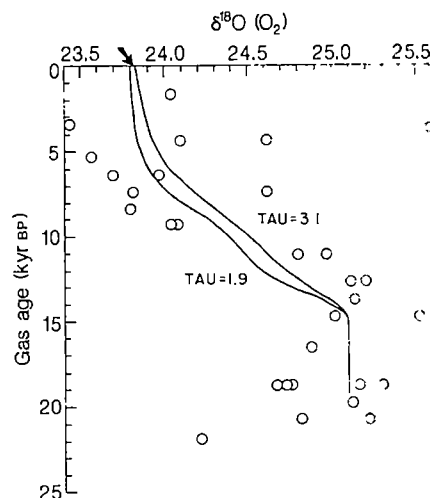


Fig. 2 Model values of atmospheric $\delta^{18}\text{O}(\text{O}_2)$ versus age BP, calculated for atmospheric O_2 turnover times of 1.9 and 3.1 kyr, along with $\delta^{18}\text{O}(\text{O}_2)$ values measured in the Dome C ice core. The modern atmospheric value is shown by the arrow. The calculations assume melting, at a constant rate, of 60% of all ice which melted during the last glacial termination, between 15.0 and 13.0 kyr BP, the remainder melting at a constant rate between 10.5 and 8 kyr BP. Surface sea water $\delta^{18}\text{O}$ is taken as 0‰ at present, and $+1.3\%$ before 15 kyr BP; it is assumed to vary proportionately to ice volume during intermediate times. Model calculations are done using the equation: $d\delta^{18}\text{O}(t)/dt = (\delta^{18}\text{O}(\text{ss}) - \delta^{18}\text{O}(t))/\text{TAU}$, where t is time, TAU is atmospheric O_2 turnover time, and $\delta^{18}\text{O}(\text{ss})$ and $\delta^{18}\text{O}(t)$ are the $\delta^{18}\text{O}$ values of atmospheric O_2 at steady state and at time t , respectively. The steady-state value is taken as $+23.5\%$ with respect to surface sea water.

change in sea water $\delta^{18}\text{O}$. This similarity may be surprising, as can be seen from considering the controls on the $\delta^{18}\text{O}$ of atmospheric O_2 . Isotopic exchange between water and atmospheric oxygen occurs through transfer of oxygen atoms within the biological pathways, by photosynthesis and respiration. At steady state, the $\delta^{18}\text{O}$ of O_2 consumed by respiration must be equal to the $\delta^{18}\text{O}$ of O_2 produced by photosynthesis. The $\delta^{18}\text{O}$ of photosynthetic O_2 is: $\delta^{18}\text{O}(\text{photosynthetic } \text{O}_2) = \delta^{18}\text{O}(\text{water}) + A$, where $\delta^{18}\text{O}(\text{water}) = \delta^{18}\text{O}(\text{ocean}) + W$, A equals the kinetic isotope effect during photosynthesis¹⁸ and W is the weighted mean $\delta^{18}\text{O}$ difference between the global sea water $\delta^{18}\text{O}$ and the $\delta^{18}\text{O}$ of the water which is the immediate source for photosynthetic oxygen. The $\delta^{18}\text{O}$ of respiratory O_2 is: $\delta^{18}\text{O}(\text{respiratory } \text{O}_2) = \delta^{18}\text{O}(\text{O}_2) + B$, where $\delta^{18}\text{O}(\text{O}_2)$ is the atmospheric O_2 $\delta^{18}\text{O}$ and B is the respiratory kinetic isotope effect^{19,20}. At steady state, $\delta^{18}\text{O}(\text{O}_2) - \delta^{18}\text{O}(\text{ocean}) = W + A - B$. All three terms on the right-hand side probably changed between glacial and interglacial times. W varied for terrestrial photosynthesis because of changing isotope effects in the hydrological cycle and in evapotranspiration. A and B (the kinetic photosynthetic and respiratory isotope effects) varied because different plants, animals and bacteria were responsible for O_2 production and consumption during glacial times. The similarity of the $(W + A - B)$ term between the last ice age and today, as inferred from our data, may reflect a very strong stability in oxygen isotope fractionation by the most productive marine and terrestrial ecosystems, and the hydrological regimes in which they lie.

The second interesting feature of the $\delta^{18}\text{O}(\text{O}_2)/\text{age}$ record concerns the shape of the curve during the last glacial termination. The rate at which the glacial steady-state O_2 $\delta^{18}\text{O}$ value ($+25.1\%$) approaches the postglacial value ($+23.8\%$) depends on the time course of change in the $\delta^{18}\text{O}$ of sea water due to deglaciation, and the lag in the isotopic equilibration of atmospheric O_2 with respect to photosynthesis. The latter term is, in turn, equal to the magnitude of the global O_2 reservoir divided by the gross rate of photosynthesis on the planet. The atmospheric O_2 turnover time is estimated to be in the range of 3.1–1.9 kyr (Table 2).

Table 2 Parameters for estimating the turnover time of atmospheric O₂

Global atmospheric O ₂ reservoir (GO ₂ R) = 3.7×10^{19} mol
Terrestrial net primary productivity (TNPP) = 5×10^{15} mol yr ⁻¹ (refs 21, 22)
Terrestrial gross primary productivity (TGPP) = $2 \times$ TNPP (ref. 23) = 10×10^{15} mol yr ⁻¹
Marine primary productivity (MPP) = 2×10^{15} (ref. 24)– 10×10^{15} mol yr ⁻¹ (refs 24, 25)
Global primary productivity (GPP) = (TGPP + MPP) = 12×10^{15} – 20×10^{15} mol yr ⁻¹
Atmospheric O ₂ turnover time = (GO ₂ R/GPP) = 3.1–1.9 kyr

In Fig. 2, results are given for simple model calculations of $\delta^{18}\text{O}$ (O₂) versus time (described in Fig. 2 legend), for the atmospheric O₂ turnover times estimated above. The comparison of the model curves with the observed variation in $\delta^{18}\text{O}$ of atmospheric O₂ suggests a lower limit on global productivity around the time of the last glacial termination: our data, although scattered, show that the decrease in atmospheric $\delta^{18}\text{O}$ to the postglacial values, initiated at the earliest around 13 kyr BP, was nearly complete by 9 kyr BP. The oxygen isotopic decrease was, therefore, at least as fast as estimated from the model, assuming a turnover time of 3.1 kyr (Fig. 2). This result suggests that productivity values during deglaciation were $\geq 12 \times 10^{15}$ mol yr⁻¹ (the low estimate for present productivity). This does not exclude the possibility that global primary productivity during the last glacial termination was less than at present, if current productivity is on the high side of the different estimates.

We conclude that, during the past 22 kyr, the $\delta^{18}\text{O}$ of atmospheric O₂ has changed along with that of sea water. The change reflects, in a complex way, the controls on the oxygen isotope fractionation between sea water and atmospheric O₂ and the rate of global primary productivity. In addition, since atmospheric O₂ is well mixed isotopically, $\delta^{18}\text{O}$ (O₂) is a time-stratigraphical marker. It can be used for the correlation between climatic records obtained from marine and ice cores. This is specially important for interpreting palaeo-CO₂ records from ice.

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Silicon coordination changes from 4-fold to 6-fold on devitrification of silicon phosphate glass

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4-Fold oxygen coordinated tetrahedral silicon (^{IV}Si) is the common building block in silicas and silicates. However, 17 crystallographically well-characterized inorganic silicates are known in which silicon exists in 6-fold oxygen octahedral coordination¹. The most notable is stishovite, a high-pressure SiO₂ polymorph^{2,3}. An interesting question is whether differences in silicon oxygen coordination between liquid and solid silicates impose a barrier to nucleation. One such case is stishovite (^{VI}Si) which cannot be synthesized from SiO₂ with ^{IV}Si at ambient pressures^{4,5}. That such an apparent nucleation barrier is not limited to silicon oxygen coordination is shown by aluminium. Thus, jadeite (NaAlSi₂O₆) with ^{VI}Al will not precipitate from its isochemical glass, in which only ^{IV}Al has been proven to exist, except when subjected to pressures > 60 kbar^{6,7}. To test if transitions of silicon coordination from 4- to 6-fold impose, in general, a barrier to nucleation, we studied the SiO₂-P₂O₅ system. X-ray diffraction studies on one of the phases in this system, SiO₂-P₂O₅, show the presence of ^{VI}Si only⁸. The aforementioned examples indicate that the silicon-phosphate glass from which this phase precipitates will also contain ^{VI}Si, as no external pressure is used. Zachariasen⁹, on the other hand, has argued against 6-fold coordination in glasses, predicting that octahedral coordination would force periodicity on the lattice, thereby disrupting the vitreous state. Our ²⁹Si MAS (magic angle spinning) NMR results show that the SiO₂-P₂O₅ glass contains only ^{IV}Si which on devitrification at ambient pressures transforms to crystalline silicon-phosphate with ^{VI}Si.

The SiO₂-P₂O₅ glasses were prepared at Corning Glass Works (Corning, New York). The glasses, which were shown to be X-ray amorphous, were devitrified at 850, 1,000 and 1,300 °C. The nature of the crystalline constituents were determined by X-ray diffraction. The X-ray pattern of the sample devitrified at 1,300 °C showed only crystals of the cubic phase of SiP₂O₇ on top of an amorphous halo. The samples devitrified at 1,000 and 850 °C contained crystals of Si₅O(PO₄)₆, SiP₂O₇ and an amorphous component¹⁰.

A single-crystal X-ray study by Tillmans *et al.*⁸ has confirmed the 6-fold coordination about Si in the cubic polymorph of SiP₂O₇ determined by earlier workers^{11,12}. They found six crystallographically distinguishable silicon sites with Si-O bonds of different lengths and a range of Si-O-P angles. All of the phosphorus exists as pyrophosphate. Si₅O(PO₄)₆ consists of isolated SiO₆ octahedra and Si₂O₇ groups with ^{IV}Si which are linked by PO₄ tetrahedra¹³. The phase diagram for this SiO₂-P₂O₅ system has been reported by Tien and Hummel¹⁴. (However, the 5SiO₂-3P₂O₅ component was incorrectly assigned^{13,15} as 2SiO₂-P₂O₅.)

²⁹Si-NMR data were collected on a Bruker CXP-300 spectrometer operating at 59.6 MHz with a MAS accessory. The magic angle was adjusted with benitoite¹⁶. The spinning rate was 2.5 kHz using an Andrew-type spinner. The chemical environments of the phosphorus atoms were also examined using ³¹P MAS NMR on a home-built 180-MHz spectrometer¹⁷ operating at 72.854 MHz.

Figure 1 shows ²⁹Si MAS NMR spectra obtained from a glass and from the same glass devitrified at the three different temperatures. The lines near -110 p.p.m. (parts per 10⁶) are characteristic of tetrahedrally coordinated Si and those around

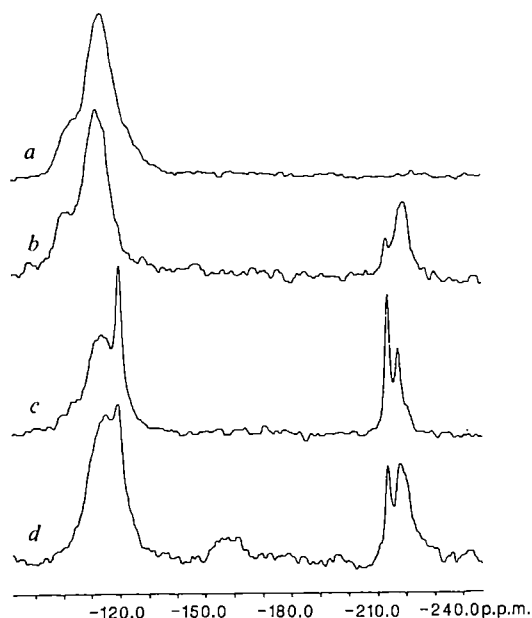


Fig. 1 ^{29}Si MAS NMR spectra from the $\text{SiO}_2\text{-P}_2\text{O}_5$ system. The chemical shift is referenced to a quartz external standard (-107.5 p.p.m.). Excitation pulses were $4\text{ }\mu\text{s}$ long, corresponding to $\sim 60^\circ$ pulses. Delay between pulses was 20 s for the glass and 1 s for the devitrified samples. Increasing the delay up to 10 min for the glass and to 5 min for the devitrified samples did increase the intensity of the octahedral lines in the devitrified samples by $\sim 25\%$ but no octahedral signal was observed in the glass. *a*, Spectrum of the glass resulting from 12,000 FID (free induction decays). *b*, Spectrum of glass devitrified at $1,300^\circ\text{C}$. The X-ray powder pattern showed the presence of SiP_2O_7 only. The spectrum is from 72,250 FID. *c*, Glass devitrified at $1,000^\circ\text{C}$ for 6 h . The X-ray powder pattern showed the presence of both SiP_2O_7 and $\text{Si}_5\text{O}(\text{PO}_4)_6$, the latter in the greater amount. 69,700 FID. *d*, Spectrum of glass devitrified at 850°C for 16 h . The X-ray powder pattern showed that crystals of SiP_2O_7 and $\text{Si}_5\text{O}(\text{PO}_4)_6$ were present in similar amounts. 12,750 FID were co-added and transformed to give the spectrum.

-215 p.p.m. indicate the presence of the octahedral coordination¹⁸. The spectrum of the glass, shown in Fig. 1*a*, and the spectra of the three different partially crystalline samples, shown in Fig. 1*b-d*, demonstrate that silicon exhibits only 4-fold coordination in the glass but, on devitrification, 6-fold coordinated silicon appears.

The X-ray powder pattern of the sample devitrified at $1,000^\circ\text{C}$ showed twice the ratio of $\text{Si}_5\text{O}(\text{PO}_4)_6\text{:SiP}_2\text{O}_7$ that was seen in the sample devitrified at 850°C . Hence, a comparison between the spectrum in Fig. 1*b* containing only SiP_2O_7 and those in Fig. 1*c* and *d*, which contain different relative amounts of both crystalline components, allows assignment of the peaks. The SiP_2O_7 (Fig. 1*b*) shows a narrow octahedral line at -214 ± 1 p.p.m. and a broad line centred at -220 p.p.m. In Fig. 1*c* and *d*, the transition at -214 ± 1 p.p.m. changes with composition, due to $\text{Si}_5\text{O}(\text{PO}_4)_6$ having ^{29}Si . The $\text{Si}_5\text{O}(\text{PO}_4)_6$ probably also contributes to the intensity at -217 ± 1 p.p.m. These chemical shift values may be contrasted with the value of -191 p.p.m. for stishovite¹⁸ and -179.5 p.p.m. for thaumasite¹⁹, the only solids containing ^{29}Si for which ^{29}Si NMR data have been collected to date. The narrow peak in Fig. 1*c* and *d* at -120 ± 1 p.p.m. is assigned to ^{29}Si in the crystalline $\text{Si}_5\text{O}(\text{PO}_4)_6$ phase.

A substantial chemical shift of the ^{29}Si occurs relative to usual Q_1 shifts: compare ^{29}Si in $\text{Si}_5\text{O}(\text{PO}_4)_6$ with a O-Si-O angle of 180° and chemical shift of ~ -120 p.p.m. with ^{29}Si in $\text{Sc}_2\text{Si}_2\text{O}_7$ also with a O-Si-O angle of 180° and chemical shift of -93.5 p.p.m. (ref. 1). This upfield shift is attributed to phosphorus. The relative intensity of the amorphous peak that is the most downfield (-103 p.p.m.) decreases more rapidly with the progress of devitrification than does the upfield peak.

The ^{31}P -NMR spectra reflect several different environments for the phosphorus nuclei. For the glass, we see five lines with chemical shifts of 15 , -0.4 , -12 , -54 and -72 p.p.m. relative to an external standard of 85% H_3PO_4 . The partially devitrified samples showed changes in the intensity of these lines and, in the multi-component cases, a new line at -44 p.p.m. which must be due to the crystalline $\text{Si}_5\text{O}(\text{PO}_4)_6$ phase. The spectra are complicated by overlapping spinning side bands due to the large chemical shift anisotropy of ^{31}P . Assignment of the peaks is also difficult due to the range of chemical shifts which have been reported for phosphates^{20,21}.

The reconstructive transitions, requiring the making and breaking of strong Si-O bonds, which change the coordination of the Si from 4-fold in the glass to 6-fold in the crystal occurs in this system at ambient pressures. The 4 to 6-fold transition does not prohibit nucleation of the crystalline phase, indeed, the phase containing only ^{29}Si appears to nucleate first. The presence of only tetrahedral coordination in the glass is consistent with Zachariasen's prediction.

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Transfer of structural information from Langmuir monolayers to three-dimensional growing crystals

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One enantiomer of an initially racemic mixture of α -amino acids can be preferentially removed from solution by selective incorporation into the opposite enantiotopic faces of growing centrosymmetric crystals of the α -form of glycine^{1,16}. We report here that oriented growth of crystals of the α form of glycine has been achieved under chiral Langmuir monolayers comprising amphiphilic α -amino acids, by virtue of a structural match between the monolayer and the *ac* surface layer of the attached growing glycine crystals. Such monolayers of α -amino acid of *R* configuration, containing long hydrocarbon chains, induce glycine to crystallize with its (010) face attached to the monolayer, and by symmetry the corresponding *S* amino-acid monolayers induce attachment of the (0 $\bar{1}$ 0) face of glycine. Replacement of the hydrocarbon by a fluorocarbon chain induces analogous crystallizations, albeit, with only a partial degree of orientation, whereas monolayers of a resolved amino acid bearing a cholestanoyl moiety do not promote crystallization of glycine. Monolayers of the *R*, *S*-amino acids

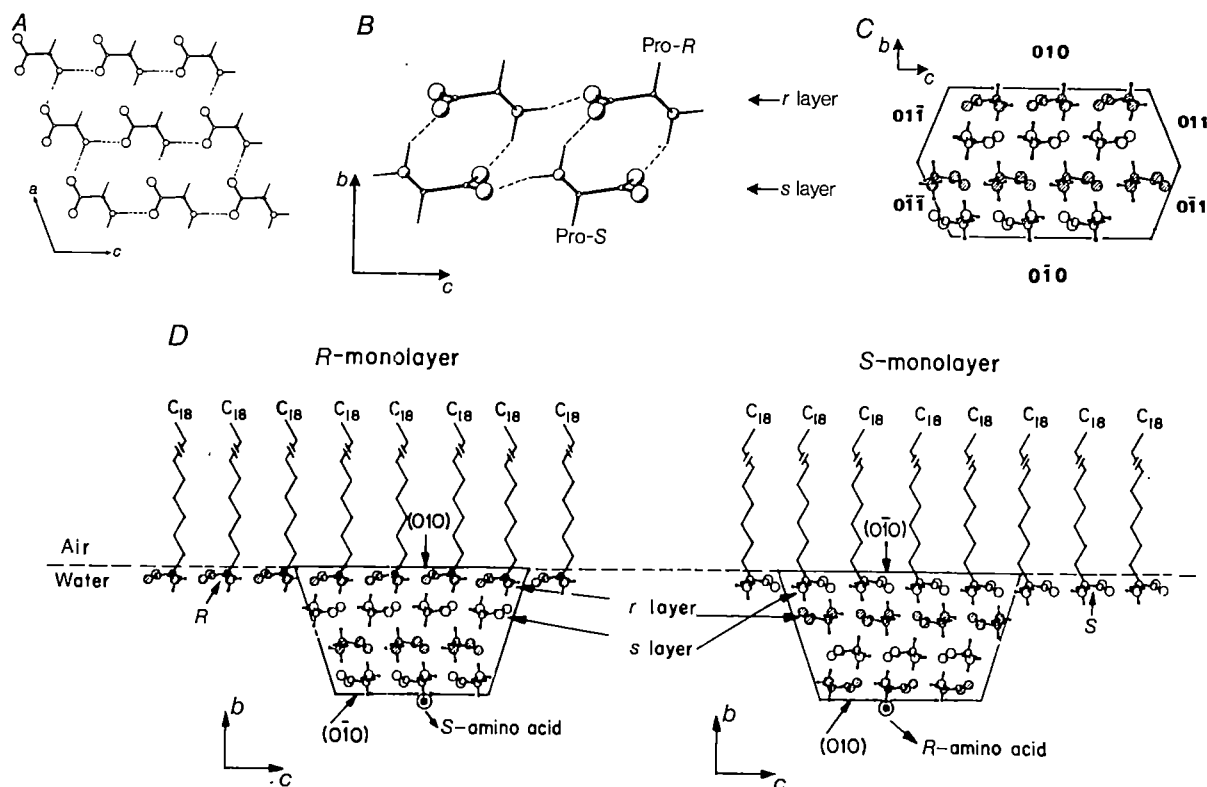


Fig. 1 A, An *ac* layer of hydrogen-bonded glycine molecules viewed along the *b*-axis. The layer is defined as *r* since the H atoms of the C—H bonds which emerge from the *ac* plane are pro-*R*. B, A centrosymmetric bilayer of hydrogen-bonded glycine molecules viewed perpendicular to the *bc* plane. The upper *r* layer is that shown in A. C, Packing arrangement of α -glycine, delineated by its crystal faces. The bilayers (B) are related by 2-fold screw symmetry along the *b*-axis. D, Schematic views of pyramidal crystals of glycine grown under compressed *R* and *S* amino-acid monolayers. The (010) face of glycine is attached to the *R* monolayer and the (0 $\bar{1}$ 0) face to the *S* monolayer. *S* and *R* amino acids are shown adsorbed from solution at the (0 $\bar{1}$ 0) and (010) faces respectively.

induce attachment of both (010) and (0 $\bar{1}$ 0) faces of glycine. These results on oriented crystal growth provide a new route for efficient amplification of optical activity of amino acids present in solution, through the enantioselective occlusion into the growing crystals of glycine¹ at water–air interfaces covered by a monolayer.

Glycine crystallizes from aqueous solution in the α -form (space group $P2_1/n$), exhibiting a bipyramidal habit¹. The glycine molecules form hydrogen-bonded chiral layers parallel to the *ac* plane² (Fig. 1A). These glycine layers (designated as *r* and *s*) are juxtaposed on one side of the *b* axis by centres of inversion about which the molecules are interlinked by N—H...O bonds into dimers to form centrosymmetric hydrogen-bonded bilayers (Fig. 1B). These bilayers are related by 2-fold screw symmetry to complete the crystal packing (Fig. 1C).

We anticipated that a densely packed Langmuir monolayer of amphiphilic homochiral α -amino acid molecules would exhibit in the aqueous subphase a hydrogen-bonded layer arrangement very similar to that in glycine, provided the hydrophobic moieties of the monolayer allow the neighbouring glycol head groups to be interlinked by N—H...O bonds. Consequently, a monolayer of, say, resolved *R*- α -amino acids packed as tightly as in an *ac* layer of glycine, should simulate an *r* layer of glycine molecules exposed at the (010) face of the crystal (see Fig. 1A, D), and thus might induce nucleation of a glycine crystal with its (010) face attached to the monolayer (Fig. 1D). By symmetry, the corresponding monolayer of *S*-amino acids should, in identical conditions, form an *s* layer and induce nucleation of a glycine crystal with its (0 $\bar{1}$ 0) face attached to the monolayer (Fig. 1D). Amphiphilic α -amino acids 1–5 (Table) were synthesized to test this hypothesis.

Condensed monolayers of compounds 1–3, measured over pure water, exhibited limiting areas per molecule in the range of 25–29 Å² (Fig. 2). These values straddle the molecular unit surface area (that is, $ac \sin \beta$) of 25.6 Å² of an *ac* layer of glycine molecules in its crystal structure. The compression isotherms of

the monolayers over aqueous solutions of glycine exhibited a distinct expanded character (Fig. 2), the effect being more pronounced with increasing concentration of glycine. This observation indicates the presence of interactions between the monolayer molecules and glycine from the subphase^{3,4}. At high compressions, all isotherms of a given compound converge (Fig. 2), indicating possible similarity in the final compressed structures of the respective films.

Monolayers 1–3 promoted immediate nucleation (within seconds after formation of the film) of pyramidal glycine crystals with their basal *ac* faces attached to the monolayer. When the *R*- α -amino acids were used, most pyramids were attached through their basal (010) faces to the monolayer and, by symmetry, when *S*- α -amino acids were used, enantiomorphous pyramids were formed with their basal (0 $\bar{1}$ 0) faces attached to the monolayer. Monolayers of racemic composition yielded attached pyramidal crystals of both types (Table 1).

With compound 5, which contains the cholestanoyl moiety, the limiting area per molecule in the monolayer is determined by the bulky steroid skeleton. It was measured to be 38 Å² (Fig. 2), a distinctly larger value than that of the glycol head group, thus precluding hydrogen bonding between the latter groups. Indeed, crystallization of glycine under such a monolayer was observed to occur at a much slower rate than with monolayers of compounds 1–3 (hours as opposed to seconds) and mostly in the bulk subphase rather than at the monolayer. In the few cases in which glycine also crystallized at the interface, these crystals exhibited both pyramidal and bipyramidal morphologies with no preferential orientation (Table 1).

For compound 4, containing a fluorocarbon chain, the situation is less clear. We might expect, with a limiting molecular surface area of the order of 30 Å² (Fig. 2)^{5,6}, that the glycol head groups would form a hydrogen-bonded layer, but with longer hydrogen-bonding distances than in the corresponding hydrocarbon monolayers. Monolayers of compound 4 of *S*

Table 1 Crystallization of α -glycine under compressed monolayers at the air-water interface

Monolayer	Limiting area per molecule (\AA^2)	Degree of orientation (%)	Face preferentially exposed to the monolayer	Rate of crystallization
1 $R\text{-CH}_3\text{-(CH}_2\text{)}_{15}\text{-CH-(NH}_3^+\text{)-CO}_2^-$ *	25	93	(010)	Fast (seconds)
$S\text{-CH}_3\text{-(CH}_2\text{)}_{15}\text{-CH-(NH}_3^+\text{)-CO}_2^-$ *	25	91	(0 $\bar{1}$ 0)	Fast
$R, S\text{-CH}_3\text{-(CH}_2\text{)}_{15}\text{-CH-(NH}_3^+\text{)-CO}_2^-$ *	25	(~50)	(010)	Fast
2 $R\text{-CH}_3\text{-(CH}_2\text{)}_{17}\text{-OCO-CH}_2\text{-CH-(NH}_3^+\text{)-CO}_2^-$ †	29	>99	(010)	Fast
$S\text{-CH}_3\text{-(CH}_2\text{)}_{17}\text{-OCO-CH}_2\text{-CH-(NH}_3^+\text{)-CO}_2^-$ †	29	>99	(0 $\bar{1}$ 0)	Fast
3 $R\text{-CH}_3\text{-(CH}_2\text{)}_{17}\text{-OCO-(CH}_2\text{)}_2\text{-CH-(NH}_3^+\text{)-CO}_2^-$ †	29	>99	(010)	Fast
$S\text{-CH}_3\text{-(CH}_2\text{)}_{17}\text{-OCO-(CH}_2\text{)}_2\text{-CH-(NH}_3^+\text{)-CO}_2^-$ †	29	>99	(0 $\bar{1}$ 0)	Fast
4 $S\text{-CF}_3\text{-(CF}_2\text{)}_9\text{-(CH}_2\text{)}_2\text{-OCO-CH}_2\text{-CH-(NH}_3^+\text{)-CO}_2^-$ ‡	—‡	65-75	(0 $\bar{1}$ 0)	Fast
5 $S\text{-5-}\alpha\text{-Cholestan-3}\beta\text{-OCO-CH}_2\text{-CH-(NH}_3^+\text{)-CO}_2^-$ †	38	No orientation	—	Slow (hours)

The molecular unit surface area of α -glycine in an *ac* layer = $ac \sin \beta = 25.6 \text{ \AA}^2$. All crystallization experiments were performed at $20 \pm 2^\circ \text{C}$ by compressing the monolayers to their limiting areas per molecule over 4.66 M aqueous solutions of glycine. The monolayer area was kept constant during crystallizations. All monolayer-forming compounds have been unambiguously characterized by spectroscopic methods (NMR, MS, IR) and elemental analysis. All areas per molecule were measured over doubly distilled water at $20 \pm 2^\circ \text{C}$. The degree of orientation is defined as the percentage of crystals exposing a given attached face, that is, (010) or (0 $\bar{1}$ 0), to the monolayer. The rate of crystallization refers to the time elapsed between the compression of the film to its final area and the visual observation of first crystals attached to the film.

* Synthesized by aminolysis of α -bromostearic acid. The racemate was resolved into enantiomers by crystallization of the brucine salts of the *N*-formyl derivatives. The enantiomeric excess was determined to exceed 96% for monolayer 1(*R*) and 90% for monolayer 1(*S*) by $^1\text{H-NMR}$ and HPLC analyses of the diastereomeric *N*-formyl aminostearoyl *S*-phenylalanine methyl ester.

† Resolved compounds 2-5 were prepared by esterification of enantiomerically pure *N*-carbobenzoxy- α -benzyl aspartic or glutamic acids with the respective alcohols. The protective groups were cleaved by hydrogenolysis on Pd/C in 90% trifluoroacetic acid. The enantiomeric purity was determined to exceed 99% by gas chromatographic analyses¹³ of the respective *N*-trifluoroacetoxy diisopropyl aspartates or glutamates obtained by acidic hydrolysis of compounds 2-5 followed by derivatization.

‡ The area per molecule could not be reproduced because of technical problems (see Fig. 2).

configuration induced immediate crystallization of glycine. However, pyramids of both orientations were formed at the interface, that is, exhibiting (010) and (0 $\bar{1}$ 0) basal faces, yet with distinct preference for the latter (Table 1).

Crystallization experiments conducted with *R* monolayers of compound 2 in a range of areas per molecule showed complete orientation of glycine crystals at the monolayer up to surface areas five times its limiting value. This result suggests the formation of aggregates of very similar structure to the compressed monolayer which act as nucleation sites. The orientational effect is gradually lost at larger surface areas. Similar experiments conducted with monolayers of compounds 4 and 5 in a range of areas per molecule (up to five times their limiting areas) showed no orientational effect. These observations seem to rule out a crystallization mechanism involving a trivial surface concentration effect.

The three distinct modes of crystallization under the monolayers, fast oriented, fast unoriented and no crystallization or slow unoriented, imply a very sensitive dependence of the crystallization process on small changes in the structure of the monolayer. The fast oriented crystallization suggests the presence of a close fit between the arrangement of the glycol head groups in the monolayer and that in the *ac* layer of the glycine crystal. Departures from such a fit, possibly in only one direction, result in a significant loss of crystal orientation, without affecting the rate of crystallization. The need for cooperativity between the glycol moieties is clearly demonstrated in the third mode of crystallization, in which the nucleation rate is strongly inhibited. The observation of the fast crystallization of glycine with both (010) and (0 $\bar{1}$ 0) faces under a racemic monolayer may be interpreted not only in terms of a spontaneous enantiomeric segregation of the monolayer molecules into chiral domains⁷, similar in structure to that of the resolved enantiomers, but also in terms of other packing motifs arising from the miscibility of the *R* and *S* molecules. A detailed understanding of the structural correlation between the monolayers and the growing crystals must await atom-atom potential energy calculations and two-dimensional X-ray diffraction experiments using total external reflection of synchrotron radiation^{8,9}.

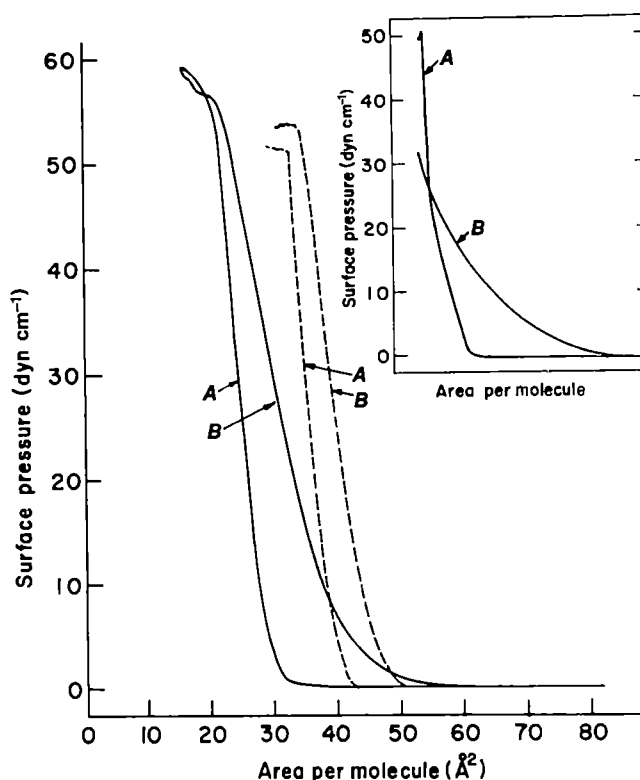
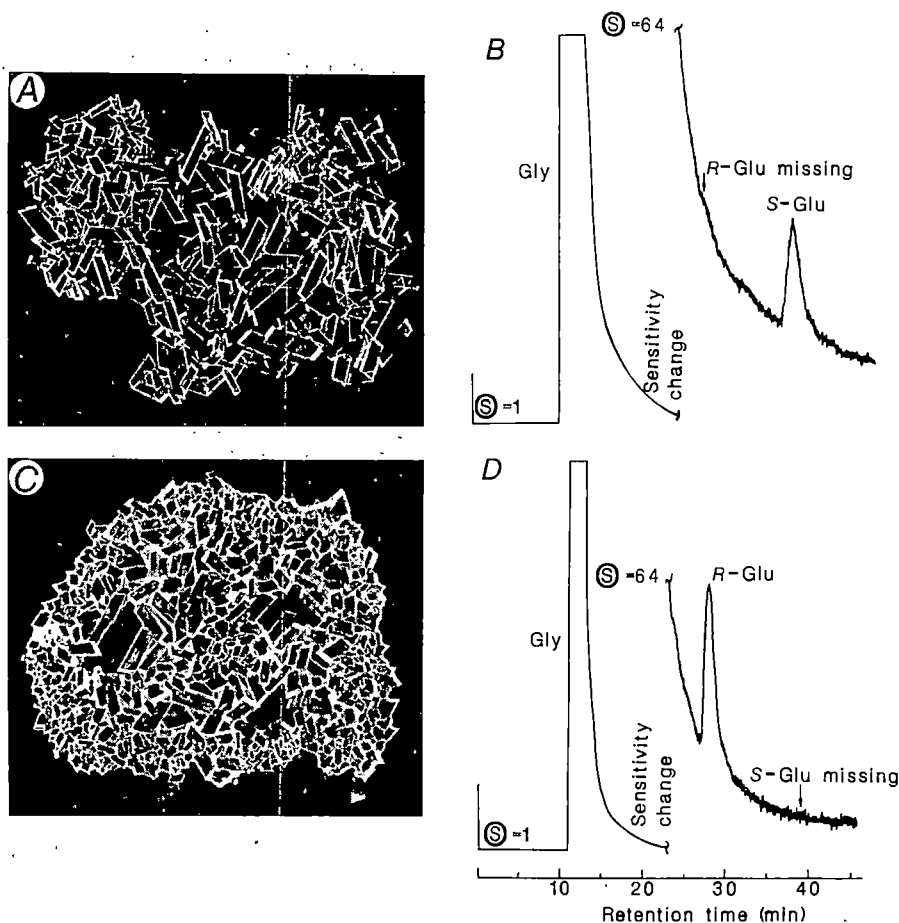


Fig. 2 Surface pressure-area isotherms measured with a thermostated circular trough equipped with a Wilhelmy balance (Mayer Feintechnik, Gottingen)¹⁴ at $20 \pm 2^\circ \text{C}$ on pure water (A) and a 2.66 M aqueous solution of glycine (B). —, Compound 2(*S*), ---, compound 5. The monolayers were spread from solutions in hexane (96%) and trifluoroacetic acid (4%). The insert displays isotherms of the fluorocarbon compound 4. The area per molecule of compound 4 could not be reproduced because of the formation of inhomogeneous spreading solutions. Expansion effects of monolayer films over salt solutions, similar to those observed here (curves B), have been reported for several systems^{3,4}.

Fig. 3 **A**, Crystals of glycine grown under a compressed monolayer of compound 2(*R*) from a 4.66 M aqueous solution of glycine containing 1% w/w *R*, *S*-Glu. All the crystals expose their (010) faces to the monolayer and exhibit the same enantiomorphous morphology. **B**, HPLC analysis of an ensemble of crystals grown as in **A** after washing with water. Conditions used: reverse-phase column (25 cm × 4.5 mm) self-packed with 5-μm Nucleosil C₁₈ (Machery Nagel). Mobile phase composition: aqueous solution of cupric acetate (4 mM) and *N,N*-dipropyl-L-Ala (8 mM) at pH 5.3–5.5 (ref. 15); post-column derivatization with *o*-phthalaldehyde and mercaptoethanol and fluorescence detection; flow rate, 0.4 cm³ min⁻¹, sensitivities \mathbb{S} as indicated¹⁵. **C**, Crystals of glycine grown under a compressed monolayer of compound 2(*S*) from a 4.66 M aqueous solution of glycine containing 1% w/w *R*, *S*-Glu. All the crystals expose their (010) faces to the monolayer and exhibit the opposite enantiomorphous morphology of those in **A**. **D**, HPLC analysis of an ensemble of crystals grown as in **C** after washing with water. Conditions as in **B**.



The ability to transfer structural information from monolayers to growing three-dimensional crystals suggests a new process for the amplification of optical activity at interfaces. It has been demonstrated previously that α -amino acids from a racemic mixture are enantioselectively occluded through the opposite {010} faces of a growing glycine crystal, leading to segregation along the *b*-axis^{1,10}. The *R*- α -amino acids are occluded only through the (010) face, whereas the *S*- α -amino acids are occluded only through the enantiotopic (0 $\bar{1}$ 0) face. Thus, when crystals of glycine were grown under an *R* monolayer of compound 2 in a solution containing racemic glutamic acid, only the *S* enantiomer was occluded into the growing glycine crystals attached to the monolayer (Fig. 3A, B), thus enriching the solution with *R*-glutamic acid. The analogous experiment performed with a monolayer of *S* configuration yielded only *R*-glutamic acid inside the glycine crystals (Fig. 3C, D), thus enriching the solution with the *S* enantiomer. The amplification effect is directly related to the number of molecular layers of the growing crystal.

The present studies are being extended to the orientational growth of other organic and inorganic crystals, also with the assistance of solid supported self-assembled^{11,12} and Langmuir-Blodgett films. This work will be reported elsewhere.

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Regime and trace-element evolution of open magma chambers

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O'Hara and co-workers^{1,2} have recently summarized some of the evidence accumulated for open-system, cyclic magmatic systems and provided useful geochemical equations for the replenishment/eruption cycles of an ideal magma reservoir. I show here that O'Hara's theory fits the situation where the volcanic eruption taps the more differentiated magma before the mixing stage. An alternative ideal model is discussed, which is applicable to the more common process of hybrid magma eruption. In mid-ocean ridge basalts, this new model may explain why large variations in incompatible elements coexist with moderate depletion of compatible elements.

Trace-element behaviour during fractional crystallization of a closed-system magma chamber is governed by the Rayleigh equation. Assuming an invariant mineralogical composition of

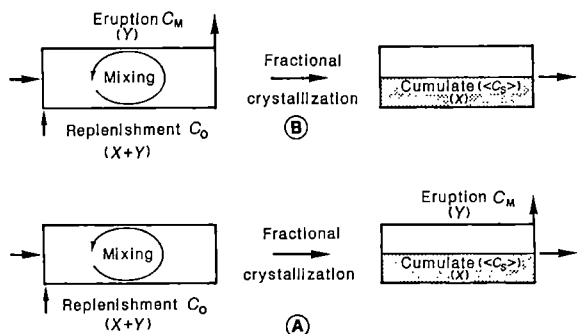


Fig. 1 The unit magmatic cycle linking replenishment, mixing, fractional crystallization and eruption for models A and B. X and Y represent the fraction crystallized and erupted in each cycle respectively. Model A is analogous to that presented by O'Hara¹ (equation (4) of the present work).

the cumulate, the concentration C_L of an element in the residual liquid is given by

$$C_L = C_{L_0} (1 - X)^{D-1} \quad (1)$$

where X refers to the mass fraction of cumulate extracted from a parent magma with concentration C_{L_0} , and D is the bulk solid/liquid partition coefficient. By mass balance, the concentration $\langle C_S \rangle$ in the bulk cumulate is given by:

$$\langle C_S \rangle = C_{L_0} \frac{1 - (1 - X)^D}{X} \quad (2)$$

O'Hara's theory describes an ideal open magma reservoir as continuously differentiating, while being periodically tapped and refilled with fresh magma which mixes homogeneously with the remaining liquid. We define C_0 as the concentration in the fresh magma, C_B as the concentration in the erupted magma, X as the fraction removed as cumulate in each cycle, whilst Y is the fraction erupted as magma during the same period. The reference is the mass of liquid which fills the chamber when replenishment resumes. At steady state, equating the elemental fluxes results in

$$X \langle C_S \rangle + Y C_B = (X + Y) C_0 \quad (3)$$

Two extreme cases will be considered that differ in their timing of eruption, replenishment and differentiation (Fig. 1):

Case A: Step 1—the reservoir is filled with a batch of fresh magma which mixes homogeneously with residual liquids from previous cycles after expulsion of some of the previous residual liquid; step 2—closed system fractional crystallization takes place within the reservoir; step 3—part of the residual liquid is expelled and the system returns to step 1.

With the present notation, $C_B = C_L$. Using equations (1) and (2), equation (3) becomes

$$\frac{C_B}{C_0} = \frac{(X + Y) (1 - X)^{D-1}}{1 - (1 - X - Y) (1 - X)^{D-1}} \quad (4)$$

which is the same equation derived previously^{1,2}.

Case B: Step 1—the reservoir is filled with a batch of fresh magma which mixes homogeneously with residual liquids from previous cycles (as in case A); step 2—part of the mixed magma is immediately erupted; step 3—closed system fractional crystallization takes place within the reservoir and the system returns to step 1.

The model differs from that derived by O'Hara in the composition C_B of the erupted magma which here equals C_{L_0} instead of C_L . Hence, equation (3) becomes

$$\frac{C_B}{C_0} = \frac{(X + Y)}{1 + Y - (1 - X)^D} \quad (5)$$

The equations for the steady-state with no crystallization ($X = 0$) are identical in both cases, whilst, as expected, they differ by a $(1 - X)^D$ term for the no-eruption limit ($Y = 0$).

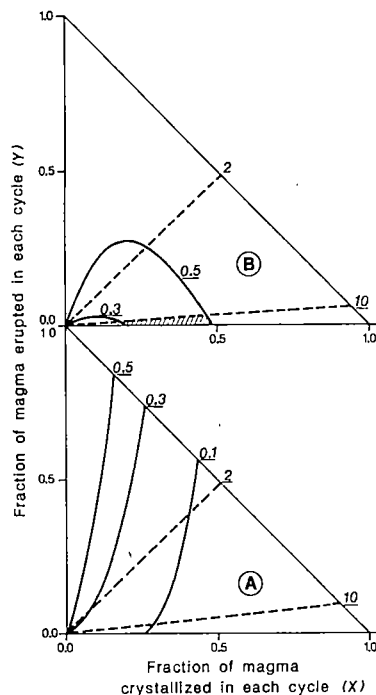


Fig. 2 Functional dependence of Y and X for a couple of elements having a bulk solid/liquid partition coefficient of $D = 5$ (solid line) and $D = 0.02$ (dashed line). Numbers underlined refer to different enrichment/depletion factors, that is, different C_B/C_0 ratios (see Fig. 1 and text for symbol explanation). The hatched area along the abscissa represents conditions of crystallization which result in both moderate depletion of transition elements and large enrichment of incompatible elements.

By analogy with the concept of residence time used in the marine environment, the characteristic number of cycles required for an element to 'approach' the cyclic concentration—say to within a few per cent—may be calculated as the ratio between the amount of this element initially present in the reservoir by the increment added in each cycle. This value, which is simply $C_B/(X + Y) C_0$ can be easily calculated from equations (4) and (5).

Both cases have a simple geological interpretation. In case A, the replenishment may be first considered as a consequence of the pressure release after the eruption of the previous cycle (Fig. 1). This sort of interpretation conflicts with geological evidence from volcanoes such as Kilauea³ or Krafla⁴: eruption is commonly preceded by a period of inflation of the chamber, which is likely to be caused by inflow of magma from below. Alternatively, eruption of a liquid may be triggered by the next pulse of fresh magma. In case A, eruption of differentiated melts implies that, for some reason (such as density contrast⁵), their mixing with the new batch of fresh magma takes place very slowly; the latter magma ponds at the base of the chamber and is involved in the eruption from the next cycle only. When mixing of high-density picritic liquids is achieved before closed system crystallization, which may not be systematic according to ref. 5, their trace-element evolution will be handled appropriately by O'Hara's equation (model A). In case B, mixing and subsequent eruption of hybrid magmas take place at the onset of the magmatic differentiation. For basaltic compositions, Sparks and co-workers⁵ predict that in many cases a turbulent mixing of fresh and differentiated magmas by the rise of plumes or diapirs occurs shortly before eruption, hence supporting model B.

Each model predicts an almost identical behaviour for incompatible elements but is extremely different for elements that are preferentially incorporated in the solid. Let us consider an element with a bulk solid/liquid partition coefficient $D = 0.02$ and an element with $D = 5$. Given an enrichment (or depletion) factor C_B/C_0 of the erupted magma relative to the fresh magma,

equations (4) and (5) may be solved for Y as a function of X . This function is plotted in Fig. 2 for cases A and B, and, for each element, assuming different enrichment (depletion) factors. As pointed out by O'Hara and Mathews², O'Hara's model (case A) cannot produce melts that have large enrichment in incompatible elements and moderate depletion of compatible elements unless both the melt fractions that crystallized and erupted in each cycle are fairly small. From field and petrological evidence, O'Hara suggests values in the range of 10^{-3} – 10^{-4} for Y and, from arguments which could also be deduced from a simple examination of my Fig. 2, concludes that X is also likely to be small.

Case B is expected to produce different trace-element patterns: inasmuch as the erupted liquid contains a significant fraction of undifferentiated fresh magma, depletion of compatible elements will not be as extreme as in case A. This model predicts the existence, along the X axis of Fig. 2, of a domain which reconciles wide variations of incompatible elements and moderate depletion of compatible elements associated with sizeable fractions of accumulated solids.

This conclusion may be illustrated using the genesis of mid-ocean ridge basalts (MORB). Although the nature of the MORB parent magma is still the subject of debate, there is some agreement that MORBs are not primitive melts which have issued from the mantle^{6,7}. Several authors have suggested that mixing fresh and residual magmas in reservoirs situated under the ridge system is an efficient mechanism for buffering the MORB composition. This mechanism would lead to characteristics of a moderately differentiated magma yet explain why primitive picritic basalts are so rare^{1,8}. Other authors have presented petrographical evidence of magma mixing and crystal/melt disequilibrium^{9–11}. Bearing in mind that some of the following assumptions are controversial, let us imagine a magma chamber which emits basalts with a Ni content of 150 parts per million (p.p.m.), typical of MORB basalts, while being periodically fed by a fresh—but not necessarily primitive—magma with 300 to 450 p.p.m. of Ni. Such fresh magma resembles the most 'primitive' basalts described on the ridges as glass of phenocryst inclusions^{10,12}. According to the present models and using the same definition as above, the Ni depletion factor would lie between 0.3 and 0.5 whilst a value of 5 would seem to be a reasonable estimate of its bulk solid/liquid partition coefficient D (ref. 13). O'Hara¹ and Bryan *et al.*¹⁴ have attributed wide variations of incompatible element concentrations in MORBs, reproducing certain features of plume-type basalts, to crystallization in an open chamber periodically replenished by new parental liquid. If an increase in the concentration of an incompatible element with $D=0.02$ (Nd, for example) by a factor 10 is required, O'Hara's mechanism requires that both the erupted and the crystallized fractions be very small in each cycle (Fig. 2a), thereby implying that almost no chemical variation could be observed among samples produced at a given locality. This inferred homogeneity is a simple consequence of a nearly perfect steady state (both X and $Y \rightarrow 0$) and is clearly opposed to the variations observed in many sites over the ridge system, both on the kilometre scale¹⁵ and among melt inclusions in closely associated samples¹⁰. Deductions from the alternative model lead to different results (Fig. 2b): taking into account O'Hara's compelling evidence that Y must be small, a 10-fold enrichment of incompatible elements consistent with 10–30% of mineral fractionation and a moderate depletion of transition elements is now possible. A consequence of this model which has not yet been fully investigated is the larger number of cycles required for high- D elements to attain steady state.

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Observations of laterally inhomogeneous anisotropy in the continental lithosphere

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Elastic anisotropy may be an important factor when seismic data are used to determine the structure, composition and dynamics of the Earth's interior^{1,2}. Azimuthal anisotropy has been found in the oceanic and, although less definitely, in the continental lithosphere from field experiments where the P_n -wave velocity was observed to vary with direction^{3–5}. These studies suggest a 180° periodicity in travel-time variations as the only criterion of the presence of azimuthal anisotropy. This criterion, however, may fail to distinguish between anisotropy and lateral inhomogeneity of the isotropic medium. A new method for measuring azimuthal anisotropy has been described recently⁶, based on the phenomenon of shear-wave splitting in anisotropic media, which suggests three criteria instead of one to identify azimuthal anisotropy. We have now applied this method to the records of permanent seismograph stations in southern Germany. Our results provide strong evidence for the presence of azimuthal anisotropy in the lithosphere of the region and reveal pronounced lateral variations in the parameters of anisotropy on a scale of about 200 km.

The method⁶ was published in Russian, so we will summarize it briefly. The idea is to analyse the transverse component T of converted shear waves like SKS or SmKS. These phases have travelled part of their path as P-waves and are therefore, in a laterally homogeneous and isotropic medium, strictly polarized as SV-waves. Should they have energy on the T component, this may be due to anisotropy or/and side refraction and scattering in a laterally inhomogeneous medium. We consider a transversely isotropic medium with a horizontal axis of symmetry, which forms a particular, relatively simple variety of azimuthal anisotropy. Such a medium is often used as a model in geophysical studies of azimuthal anisotropy. The wave fields set up in this model were examined using the Thomson–Haskell matrix method extended for anisotropic media^{7,8} and the following results were obtained.

If a plane SV-wave from an isotropic half-space passes from below into a plane horizontal, homogeneous, transversely isotropic layer with a horizontal axis of symmetry, then the incoming wave splits into two quasi-shear waves. Under realistic assumptions of the properties of the Earth's mantle (magnitude of anisotropy is of the order of several per cent, anisotropic layer thickness is of the order of 100 km, and the angle of incidence of the SV-wave is of the order of 10°), the time delay δt between the two quasi-shear waves at the top of the layer is of the order of a fraction of a second. For periods around 10 s, we may assume that $\omega \cdot \delta t \ll 1$, where ω is the circular frequency. Then, at the top of the layer, the quasi-shear waves interfere either constructively (in the radial component R) or destructively (in the transverse component T). The properties of the R

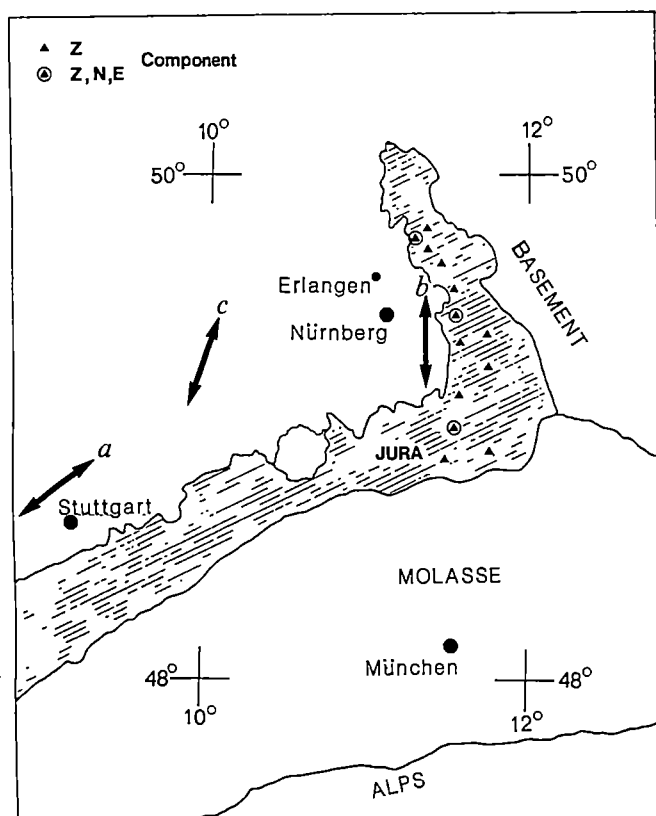


Fig. 1 Location map of the seismograph stations used and the major tectonic units of the area in southern Germany. The vertical components of the Gräfenberg array are marked by triangles, horizontal components by circles. A three-component station is located at Stuttgart. The arrows mark the directions of the fast velocity of the anisotropy observations. They are 50° at Stuttgart (a) and 0° at the Gräfenberg array (b). An averaged direction of 20° (c) results from a study of many P_n -arrival times over the entire area of southern Germany⁵. Our study indicates that the general anisotropy of the area appears to break up into smaller units if a method with higher spatial resolution is used.

component are weakly dependent on the azimuth of propagation of the incoming wave, while major variations occur in the T component.

If the angle between the azimuth of the wave propagation and the axis of symmetry is 0, $\pi/2$, π or $3\pi/2$, the T component is equal to zero. If the angle is $\pi/4$ or $5\pi/4$ and the R component of the incoming wave is represented as $2 \cos(\omega t)$, then R and T at the top of the layer can be expressed in first approximation by

$$R(t) \approx \cos(\omega t) + \cos(\omega t + \omega \delta t) \approx 2 \cos(\omega t)$$

$$T(t) \approx \cos(\omega t) - \cos(\omega t + \omega \delta t) \approx (\omega \delta t) \sin(\omega t)$$

These equations mean that the harmonic T component is shifted in time with respect to the R component by a quarter of a period, while the amplitude of the T component is proportional to frequency. The equations are also valid for the angles $3\pi/4$ and $7\pi/4$, but the expression for T changes sign. This change implies that the azimuthal variations of T are periodic with the period of π . The conclusions thus derived are in general corroborated by the calculation for other angles, while some minor complications resulting from sparse and noisy observational data may be neglected.

In summary, the long-period, harmonic T component of the displacement set up in the model considered is characterized in first approximation by the following properties⁶: (1) It is periodic as a function of the azimuth of the wave propagation; the dominant period is 180°. (2) It is shifted in time with respect to the radial component R by a quarter-period. (3) The T/R amplitude ratio is inversely proportional to the period.

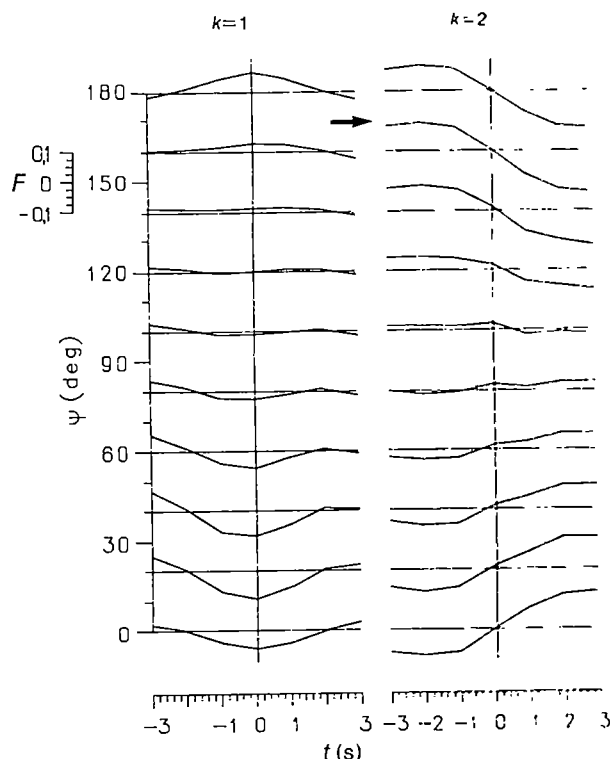


Fig. 2 Plots of $F(t, k, \psi)$ for the records of STU (modified from ref. 6). The largest deviations of $F(t)$ from 0 are observed in the right column ($k=2$) on the two top traces (marked by an arrow). These traces can be well approximated by the function $-0.12 \sin(2\pi t/10)$.

Thus, this approach suggests three independent diagnostic properties of azimuthal anisotropy instead of one in the conventional method. If all of them are observed, the probability is high that the observed effects are due to azimuthal anisotropy.

In order to extract the diagnostic properties of azimuthal anisotropy from seismic data, many earthquake recordings of the same seismograph station must be processed jointly. The combined processing of records of events with different magnitudes and different source signals requires standardization of the records. The normalized transverse component $\hat{T}(t)$ is computed for that purpose:

$$\hat{T}(t) = \int_{t_1}^{t_2} T(t+\tau) R(\tau) d\tau / \int_{t_1}^{t_2} (R(\tau))^2 d\tau$$

where the times t_1 and t_2 correspond to the beginning and end of the converted wave train and τ is the variable of integration. The use of $\hat{T}(t)$ instead of the transverse component $T(t)$ itself makes all the earthquakes directly comparable.

As an additional tool for the analysis of the azimuthal dependence, a harmonic analysis is performed

$$F(t, k, \psi) = \sum_i \hat{T}_i(t) \cos(k\varphi_i + \psi) / \sum_i (\cos(k\varphi_i + \psi))^2$$

where \hat{T}_i corresponds to the i th seismic event, φ_i is the back azimuth of the epicentre of the i th event, k is the harmonic number and ψ varies in the range from 0 to 180°. The phase ψ_0 of the k th harmonic is given by the value of ψ , which corresponds to the maximum value of $|F(t, k, \psi)|$. Denoting by t_0 the value of t which corresponds to this maximum, we may define the amplitude of the k th harmonic as $|F(t_0, k, \psi_0)|$. Obviously the values of t_0 and ψ_0 are different for different harmonics. The minimum values of $|F(t, k, \psi)|$ correspond to $\psi = \psi_0 \pm \pi/2$ and can be used to locate ψ_0 .

$F(t, k, \psi)$ can now be examined for the three properties related to azimuthal anisotropy. The 180° periodicity of the T component is observed if $|F(t_0, 2, \psi_0)|$ is greater than $|F(t_0, 1, \psi_0)|$. The property of the phase shift between $R(t)$ and $T(t)$ is observed if the shape of $F(t, 2, \psi_0)$ is close to the sine function

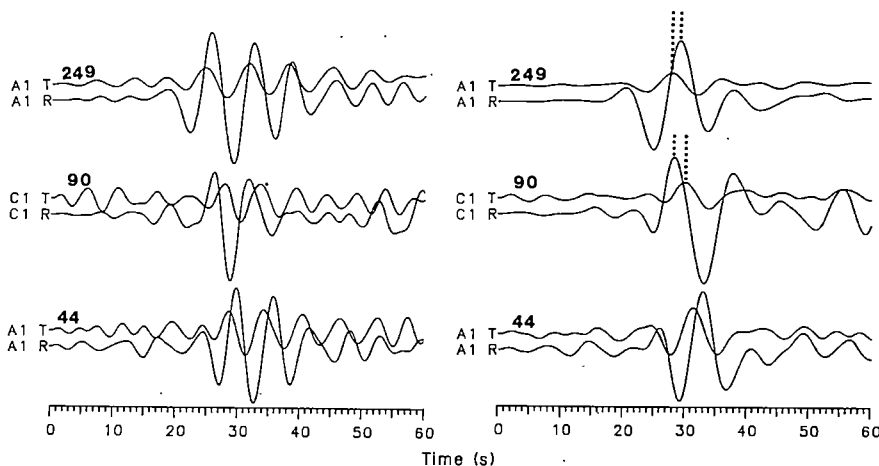
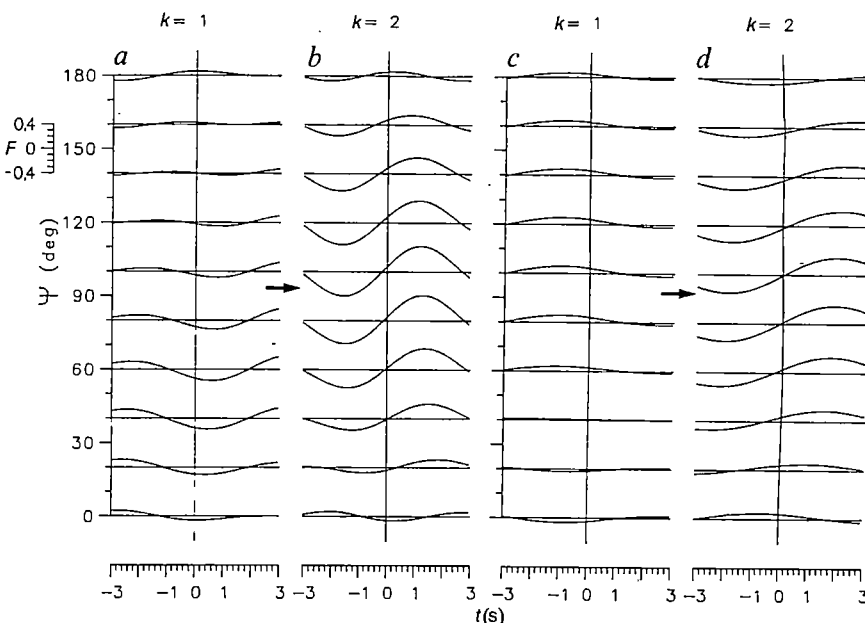


Fig. 3 SKS records of events 2, 15 and 8 of Table 1. The *R* and *T* components of these events are shown after passing through a shorter (left) and a longer (right) period bandpass filter. The numbers on the traces indicate back azimuths of the events in degrees. The letters *A* and *C* are the symbols for different sub-arrays. Note the phase shifts between the components (dotted lines).

Fig. 4 Plots of $F(t, k, \psi)$ for the records of GRF. A 4–8-s bandpass is used for *a* and *b*; and a 8–15-s bandpass for *c* and *d*. The largest deviations of $F(t)$ from 0 occur on the traces corresponding to $k=2$ and the values of ψ around 90° (marked by arrows). These traces can be well approximated by $0.4 \sin(2\pi t/6)$ (see *b*) and $0.3 \sin(2\pi t/8)$ (see *d*). The smallest values of $|F(t, 2, \psi)|$ correspond to the values of ψ around 0 and 180° conforming to $\psi_0 = 90^\circ$. Note the differences between the values of $F(t, 2, \psi)$ at GRF and STU (Fig. 2). They indicate pronounced differences in the parameters of azimuthal anisotropy.



$\sin(\omega t)$ or $-\sin(\omega t)$. The third property is observed if the values of $F(t_0, 2, \psi_0)$ are inversely proportional to the period. This requires, of course, that records in different frequency bands are available.

The direction of the fast velocity α (measured clockwise from north) can be found from the expression

$$\alpha = -\psi_0/2 + 3\pi/4$$

This expression was given in ref. 6 on the assumption that the shape of $F(t, 2, \psi_0)$ is close to $-\sin(\omega t)$. It may happen, however, that it is close to $\sin(\omega t)$. Then, instead of ψ_0 , one should use $\psi_0 + 180^\circ$.

The records of SKS and SKKS from the seismograph station STU (Stuttgart, Fig. 1) were available in the form of particle-motion plots for events of the years 1949–55 (ref. 9). It was impossible to filter these data, and the dominant periods were close to 10 s. The resulting plots of $F(t, k, \psi)$ (see Fig. 2) show that, for $k=2$, ψ_0 is close to 160 – 180° , $|F(t_0, 2, \psi_0)|$ is close to 0.12 and the diagnostic properties of azimuthal anisotropy are clearly observed: (1) The amplitude of the second harmonic $|F(t, 2, \psi_0)|$ is larger than $|F(t, 1, \psi_0)|$, implying that the influence of anisotropy is stronger than that of lateral inhomogeneity. (2) The shape of $F(t, 2, \psi_0)$ is close to the sine function, indicating the proper phase shift between the *R* and *T* components. The direction α is close to 50° . On the assumption of 7% anisotropy, the thickness of the anisotropic layer was estimated as 50 km (ref. 6).

The method was further applied to the digital broadband records of GRF (Gräfenberg array, Fig. 1; a description of this installation is given in ref. 10). The first step in processing the

records was to form beams of the radial and transverse components of the three-component stations of the array. The analysis was carried out in two frequency bands, one between 4 and 8 s and the other between 8 and 15 s. A list of the events used is given in Table 1.

Figure 3, showing examples of the filtered seismograms, indicates that considerable energy is present in the *T* component, which can only be caused by anisotropy or lateral inhomogeneity. Also clearly observable is the phase shift between the components, which can only be due to anisotropy. The properties of the plots of $F(t, k, \psi)$ (see Fig. 4) are in perfect agreement with those theoretically predicted for azimuthal anisotropy, namely: (1) the second harmonic ($k=2$) is much stronger than the first one; (2) the shape of the curve $F(t, 2, \psi_0)$ (ψ_0 is nearly 90°) is close to $\sin(\omega t)$; (3) the amplitudes of the second harmonic (0.4 for the shorter periods and 0.3 for the longer periods) are inversely proportional to the periods.

The observations at GRF provide decisive evidence for azimuthal anisotropy. The parameters of anisotropy, however, are different from those found at STU. The value of α at GRF can be estimated as 0° and the magnitude of the effect of anisotropy is twice as large as that found at STU. The difference between the values of α at GRF and STU is 50° , which is larger than the possible errors of measurement by an order of magnitude. This difference implies that the observed anisotropy is characteristic of the lithosphere and, perhaps, the top of the asthenosphere, since lateral variations of anisotropy on a scale of about 100 km in the deeper parts of the mantle would be averaged out by the summation procedure. We note that our estimates of α are close to those found from the teleseismic

Table 1 Events used in this study

Event	Date	Coordinates	Azimuth	Phase
1	25 Nov 76	19.5S 177.7W	17,197	SKKS, SKKS
2	30 Nov 76	20.6S 68.9W	249	SKS
3	21 Jul 77	53.8S 158.8E	114	PKS
4	22 Oct 77	28.0S 62.8W	240	SKS
5	17 Jul 78	15.0S 175.8W	12	SKKS
6	21 Aug 78	47.5S 32.3E	166	SKS
7	14 Mar 79	17.8N 101.3W	298	SKS
8	18 May 79	24.1N 142.4E	44	SKS
9	25 Jun 79	5.0S 145.6E	56	SKS
10	17 Oct 79	18.5N 145.4E	44	SKS
11	20 Oct 79	8.4S 115.8E	84	SKS
12	22 Oct 79	0.1N 126.0E	70	SKS
13	11 Dec 79	29.0N 141.0E	42	SKS
14	12 Dec 79	1.6N 79.3W	272	SKS
15	16 Apr 80	8.1S 108.8E	90	SKS
16	20 Jul 80	17.9S 178.6W	18	SKKS
17	11 Nov 80	51.5S 29.0E	169	SKS, SKKKS
18	4 Sep 81	9.9N 124.0E	66	SKS
19	28 Sep 81	29.4S 179.0W	25,205	SKKS, SKKKS
20	25 Oct 81	18.2N 102.0W	299	SKS
21	7 May 82	60.7S 20.8W	197	PS
22	11 Jul 83	60.9S 52.9W	211	SKS, SKKS
23	4 Oct 83	26.6S 70.8W	247	SKS
24	16 Nov 83	19.4N 155.5W	347	SKS, PS
25	22 Nov 83	0.4N 79.9W	271	SKS

Two phases were used for some events. Phases travelling in opposite directions across the globe have also been used in two cases.

P-wave travel-time residuals¹¹. The study of P_n -velocities on profiles crossing the same area in many directions has revealed a high velocity at the top of the mantle in an azimuth of 20° (ref. 5). This direction appears as the average of the substantially different directions at GRF and STU. The change of the direction of the high velocity between GRF and STU conforms to the shape of the strip of the outcrops of Jurassic rocks (Fig. 1). This conformity is either a coincidence or, more likely, the result of a relationship between the deep and shallow structure in the lithosphere.

The pronounced variations in the parameters of anisotropy within a small area imply that continental anisotropy is a relatively small-scale phenomenon compared with oceanic anisotropy. This difference is not surprising in view of the long and complicated history of the continental lithosphere. The term 'laterally inhomogeneous anisotropy' gives a proper description of the situation.

Finally, we note that the method⁶ has several advantages over conventional P_n -techniques. It does not require expensive field experiments and, as shown here, can provide higher lateral resolution. Moreover, it takes full advantage of the phenomenon of shear-wave splitting, which may be regarded as the most convincing evidence for the presence of anisotropy. Taking this into account, we recommend the method⁶ for widespread mapping of azimuthal anisotropy in the continental lithosphere. Data from dense, perhaps portable, broad-band seismograph networks would be ideally suited for this purpose.

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Near-synchronicity of New Zealand alpine glaciations and Northern Hemisphere continental glaciations during the past 750 kyr

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The Brunhes magnetochron (0–730 kyr) at Deep Sea Drilling Project Site 594 off eastern South Island, New Zealand, comprises up to ~100 m of alternating units of pelagic and hemipelagic ooze formed during interglacial and glacial periods respectively. The youngest hemipelagic interval corresponds to glacial isotope stage 2 and can be correlated with extensive glacial and glacio-fluvial deposits of the late Last Glaciation (~27–13 kyr BP) on South Island. Inferring the same genetic link for the other hemipelagic sediment units down core, we show here that as many as 12 major episodes of expanded alpine glaciation have occurred on South Island in the past 730 kyr. Each of these glacial episodes coincided with a period of significant ¹⁸O enrichment (+2‰) in the core resulting from changes in ocean isotope composition associated with major growth phases of the Northern Hemisphere ice sheets. The inferred history of alpine glaciations on this remote South Pacific island appears to be closely phase-locked (±3 kyr) to world-wide climate changes.

Site 594 of the Deep Sea Drilling Project Leg 90 is located on the southwestern margin of the Chatham Rise, 300 km east of New Zealand's South Island, in a water depth of 1,204 m (Fig. 1). The site lies in the sub-antarctic water mass immediately south of the Subtropical Convergence. The upper 170 m of the section was drilled with 95% recovery using an hydraulic piston corer¹ and provided largely undisturbed cores comprising conspicuously alternating units of light-coloured pelagic and dark-coloured hemipelagic oozes of Pliocene–Quaternary age^{2,3}. Seismic profiles⁴ demonstrate that this sedimentary sequence is

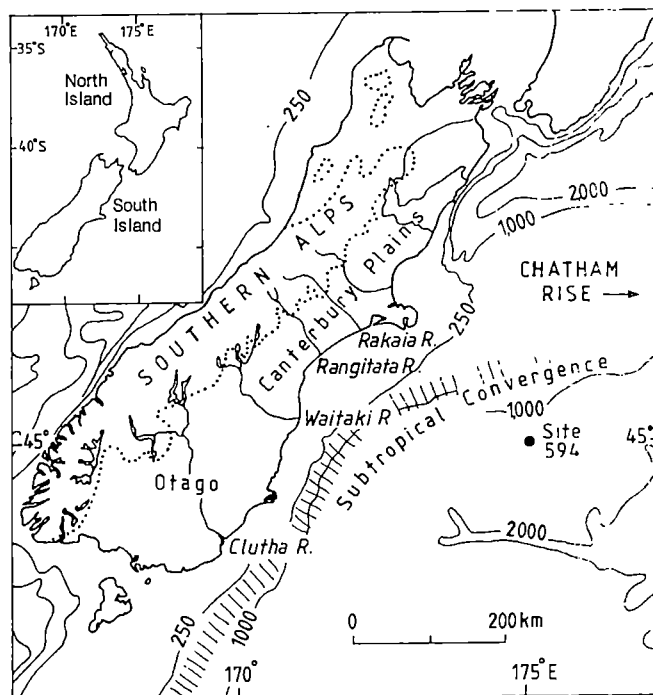


Fig. 1 Location map for DSDP Site 594 (45°31.41'S, 174°56.88'E) off South Island, New Zealand, showing also the approximate position of the oceanic Subtropical Convergence and some relevant physiographical features on South Island. The dotted line on land shows the probable extent of glacial ice at about 18 kyr BP (ref. 16).

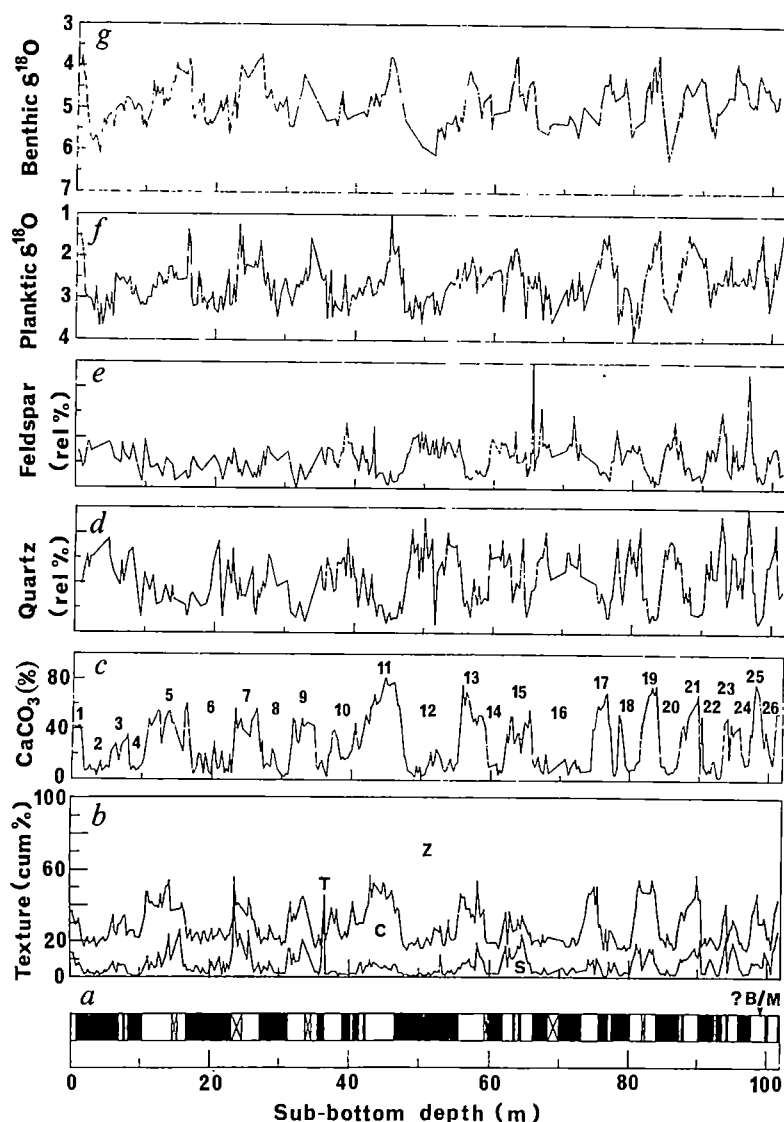


Fig. 2 Colour (a), texture (b), calcium carbonate (c), quartz (d), plagioclase feldspar (e) and planktonic and benthic foraminiferal oxygen-isotope (f and g respectively) records for the late Quaternary section of Site 594 to the Brunhes-Matuyama (B/M) boundary (730 kyr), tentatively placed at about 99.4 m sub-bottom depth³, although other options are possible. White- and black-coloured intervals (a) are dominated by pelagic and hemipelagic oozes respectively; crossed intervals represent missing core. The mean age resolution of sample points in most plots is ~2.4 kyr. In the cumulative percent textural plot (b): S, sand (>0.06 mm); C, clay (<0.004 mm); Z, silt (0.004–0.06 mm); T, tephra layer. Numbers 1–26 in the carbonate record (c) refer to major intervals of high (odd numbers) and low (even numbers) calcium carbonate content. Note that compared with the light-coloured pelagic ooze intervals the dark-coloured hemipelagic sediment units are characterized by depletion in sand and clay sizes, low carbonate contents, relatively increased amounts of quartz and feldspar, and significant ^{18}O enrichment, properties consistent with their formation during full glacial episodes. Analytical procedures and data are recorded in refs 5, 6.

regionally extensive off eastern central South Island. Here we discuss only the late Quaternary interval of core, which is complete and exceptionally thick, the Brunhes normal magnetochron (0–730 kyr) possibly extending to about 99.4 m sub-bottom depth³.

The pelagic sediments are mainly impure foraminifer-bearing nanofossil oozes whose relative enrichment in both sand- and clay-sized material (Table 1) reflects their increased proportions of foraminiferal tests and coccolith plates respectively. The hemipelagic sediment units are more variable in composition, but are typically nanofossil-, diatom- and/or sponge spicule-bearing terrigenous clayey silts with a preponderance of silt-sized quartz, sodic plagioclase, chlorite and mica minerals (Table 1).

The bulk sediment texture, calcium carbonate content and relative abundance (from X-ray diffraction) of quartz and feldspar have been determined for 315 samples collected at 30 cm intervals through the section^{5,6}, equivalent to a mean age resolution of one sample per 2.4 kyr over the past 750 kyr. These sedimentary parameters exhibit strong in-phase cyclical fluctuations down core (Fig. 2a–e). Using the carbonate stratigraphy as a basis, 26 major sedimentary alternations occur in the top 100 m of core (Fig. 2c), 13 of predominantly pelagic character (odd numbers 1 to 25) and 13 with mainly hemipelagic properties (even numbers 2 to 26).

Oxygen-isotope measurements were made on both planktonic (*Globigerina bulloides*) and benthic (mainly *Uvigerina* sp.) foraminifera from the same samples used for the sedimentary analyses⁵ (Fig. 2f, g). Despite superimposed high-frequency

fluctuations, the isotope curves display overall in-phase cyclical variations of about 2‰ in ^{18}O enrichment and depletion which in turn match the sedimentary fluctuations corresponding to the hemipelagic and pelagic ooze units respectively. Such extreme variations in the isotope composition of the ocean, as recorded in the foraminiferal tests, are controlled primarily by changes in global ice volume^{7,8} and clearly associate the hemipelagic sediments with glacial conditions and the pelagic ones with interglacial periods.

This strong interrelationship between climate and sedimentation suggests that the abundant terrigenous material in the hemipelagic deposits resulted primarily from greatly increased rates of erosion on the South Island during glacial periods and the rapid transferral of this terrigenous detritus to off-shore areas where sedimentation rates increased and the carbonate content of bottom sediments was diluted (Table 1). Direct evidence for greatly increased rates of erosion and supply of sediment from South Island comes only from the nature and distribution of on-land^{9,10} and off-shore^{11,12} deposits associated with the last major glacial advances in New Zealand, between ~27 and 13 kyr BP (ref. 13). This timing matches closely isotope stage 2 (ref. 8), which is clearly represented by the hemipelagic deposit between ~1.5 and 5.5 m sub-bottom depth in our core (unit 2 in Fig. 2c), and is discernible also in other cores from the region¹¹. At this time the lowlands of South Island east of the Southern Alps accumulated thick deposits of greywacke and schistose gravels and sands^{14–16}, commonly in the form of extensive coalescing outwash fans, such as Canterbury Plains (Fig. 1). The major eastward-draining South Island rivers also

Table 1 General sediment properties of the pelagic and hemipelagic oozes in the late Quaternary section at DSDP Site 594

Property	Pelagic oozes	Hemipelagic oozes
Colour	Light blueish-grey	Dark greenish-grey
Bulk sediment grain size		
% Sand (>63 μm)	10–30	0–10
% Silt (4–63 μm)	50–70	70–90
% Clay (<4 μm)	25–30	10–25
Calcium carbonate (%)	40–80	0–25
Nannofossils (%)	Abundant (40–75)	Rare to common (1–25)
Foraminifers (%)	Common (5–25)	Rare (1–5)
Siliceous microfossils (%) [*]	Absent to rare (0–10)	Rare to common (5–30)
Terrigenous minerals (%) [†]	Rare to common (10–40)	Abundant (30–75)
Sedimentation rates (cm kyr^{-1}) ⁵	5–10	10–35

^{*}Includes sponge spicules, diatoms, radiolarians and silicoflagellates.

[†]Includes quartz, plagioclase, chlorite and micas.

generate high proportions of suspended load^{17,18}. For example, 94% of the quartzofeldspathic chlorite schist supplied originally as bedload to the Clutha River (Fig. 1), a major source of the marine sediment off Otago and south Canterbury^{12,19}, is abraded to silt and finer sizes during its course to the sea¹⁷. During glacial periods the reduced shelf width accompanying lowered sea levels undoubtedly enhanced deep-water mud deposition^{11,12}. Moreover, the occurrence of widespread deposits of loess of Last Glacial age in east Canterbury and Otago²⁰, and the significant increase in the content of aeolian quartz in 18–15-kyr-old deep-water sediments east of New Zealand compared with their modern counterparts^{21,22} suggest that a significant proportion of the terrigenous silt in the hemipelagic sediments at Site 594 may be of aeolian origin, picked up from the extensive outwash surfaces on eastern South Island and transported offshore by the prevailing westerly wind system, which was intensified during glacial times¹³.

Thus hemipelagic unit 2 at Site 594 is the off-shore sedimentary result of climatic deterioration associated with the latest phase of extended alpine glaciation in South Island, and is directly correlatable with isotope stage 2 reflecting the last period of major expansion of the Northern Hemisphere ice sheets. The similar nature of the hemipelagic sediment intervals throughout the section (Fig. 2) strongly suggests that every hemipelagic unit reflects a major period of increased erosion and outwash aggradation associated with expanded glaciation in South Island. Moreover, because each hemipelagic interval corresponds to a period of significant (+2%) ¹⁸O enrichment (Fig. 2f, g) reflecting mainly changes in the isotope composition of the ocean due to an increase in the volume of global ice, then each episode of alpine glaciation coincided with a world-wide period of glacio-eustatically lowered sea level. The late Quaternary marine oxygen-isotope changes have been controlled primarily by the growth and retreat of continental ice sheets in the Northern Hemisphere^{7,23}; an accord between the marine ¹⁸O record and the northern European glacial record inferred from loess sequences has been demonstrated by Kukla²⁴.

That every major ice growth phase indicated by our oxygen-isotope data (Fig. 2f, g) can be closely matched through the hemipelagic units to a period of expanded alpine glaciation on a small temperate-latitude island in the Southern Hemisphere is evidence that the latter does not reflect simply local or isolated climatic events, but rather world-wide climate change. Moreover, since no consistently discernible phase difference is evident between the isotope and lithological records (Fig. 2), the climatic and sedimentary response in New Zealand to fluctuations in the world's continental ice sheets has been rapid, within the 2–3-kyr period represented by our sample spacing.

Finally, it is unfortunate that some uncertainty surrounds the exact position of the Brunhes–Matuyama boundary in this high-

resolution record²⁵, the location chosen here (Fig. 2a) being that adopted in Leg 90 of the *Initial Reports of the Deep Sea Drilling Project*^{3,5,25,26}. If this placement is correct, and the Brunhes sedimentary record at Site 594 is genuinely free of unconformities, then there have been 12 full glacial periods and 13 major interglacial episodes over the past 730 kyr (Fig. 2c), more than is implied by the standard 19 oxygen-isotope stage scheme for this time interval⁸. However, it might be argued from the shape and amplitudes of the isotope and carbonate records in Fig. 2 that the Brunhes–Matuyama boundary lies nearer 84 m sub-bottom (W. F. Ruddiman, personal communication, 1985), and that the first 19 sedimentary units defined here (Fig. 2c) match the recognized number of isotope stages for the Brunhes magnetochron. This important chronological aspect of the late Quaternary climate record at Site 594 remains to be resolved and is under investigation.

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First Mesozoic mammal from Australia—an early Cretaceous monotreme

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Here we describe Australia's first known Mesozoic mammal and the first known early Cretaceous mammal from Gondwanaland. *Steropodon galmani* n. gen. and sp., discovered in early Cretaceous sediments at Lightning Ridge, New South Wales, Australia, appears to represent an ornithorhynchid-like monotreme. This

discovery represents the first record of a fossil mammal from Australia that is older than 22.4 ± 0.05 Myr^{1,2} and the specimen is, by more than 85 Myr, the oldest known monotreme. As the oldest monotreme, it will necessitate a radical revision of present understanding about dental homology in the middle Miocene *Obdurodon insignis*, the only fossil monotreme previously known to have had teeth³. The structure of *S. galmani* supports one current view⁴ that monotremes, one of three groups of living mammals (the other two being marsupials and placentals), are phylogenetically close to the other groups of living mammals.

The opal-bearing sediments at Lightning Ridge consist of a suite of siltstones and poorly sorted sandstones referred to the early Cretaceous Griman Creek Formation⁵. In New South Wales this formation includes the Wallangulla Sandstone Member⁶, the unit from which the new monotreme was obtained. The Griman Creek Formation is dated as middle Albian, based on palynological evidence⁹. This age is supported by the discovery in the formation of hypsilophodontid dinosaurs that are very similar to those found in the Strzelecki Group of Victoria, which has been fission-track-dated as Aptian-Albian⁷. At Lightning Ridge the Griman Creek Formation was apparently deposited in the shallow estuary of a westward-flowing river. Some of the formation's invertebrate fossils are clearly marine but others, including viviparid snails, indicate a freshwater influence. Cross-bedding is present, as are apparent impressions of plant roots^{5,8}. Many of the tetrapod bones indicate transport. In addition to *S. galmani*, the vertebrate fauna from Lightning Ridge consists of teleosts, ceratodontid lungfish (*Ceratodus wollastoni* and *Neoceratodus forsteri*), turtles, plesiosaurs, crocodilians ('*Crocodylus*' *selaslophensis*), and theropod (*Rapator ornitholestoides*), ornithomimid (*Fulgurotherium australe*) and sauropod dinosaurs^{6,10,11}. There is no evidence that any of the fossils represent an intrusion of later material into the Griman Creek Formation.

The dental terminology¹² used here for *S. galmani* assumes that the teeth are tribosphenic and the cusps are homologues of topographically analogous cusps in therian mammals.

Class Mammalia

Subclass Monotremata

Family ?Ornithorhynchidae

Genus and species: *Steropodon galmani* n. gen. and sp. (Figs 1, 2).

Etymology: *Sterope* is Greek for 'flash of lightning' and *odon* is Greek for 'tooth', the name being an allusion to Lightning Ridge, the type locality, as well as to the opalescent nature of the fossil teeth.

Holotype: Australian Museum Palaeontology collection no. AM F66763, a right dentary fragment with M₁₋₃ in place, obtained from an opal miner by David and Alan Galman in 1982 or 1983. This specimen is, like all fossils from this locality, a natural opal pseudomorph of the original. Accordingly, there is no preservation of the internal bone or tooth structure although cavities in the original, such as alveoli and the mandibular canal, have been differentially preserved. The teeth preserved in the holotype have been interpreted as M₁₋₃ because the alveolus anterior to the anteriormost molar is very small. This indicates that there was an abrupt change in shape between M₁ and the tooth presumed to be a premolar situated anterior to it.

Locality: Lightning Ridge, New South Wales, Australia.

Stratigraphy: Wallangulla Sandstone Member of the Griman Creek Formation.

Age: Albian⁹.

Diagnosis: This taxon differs from all other monotremes (species of *Obdurodon*, *Ornithorhynchus*, *Tachyglossus* and *Zaglossus*) in its retention of a cristid obliqua and talonid basin and in its possession of a pre-entocristid. It differs from non-monotrematous mammals in its combination of the following features: three lower molars, an antero-posteriorly very compressed trigonid, a transverse metacristid, a well-developed and wide talonid, a pre-entocristid/cristid obliqua/distal metacristid conjunction, absence of a paraconid on M₁, absence of

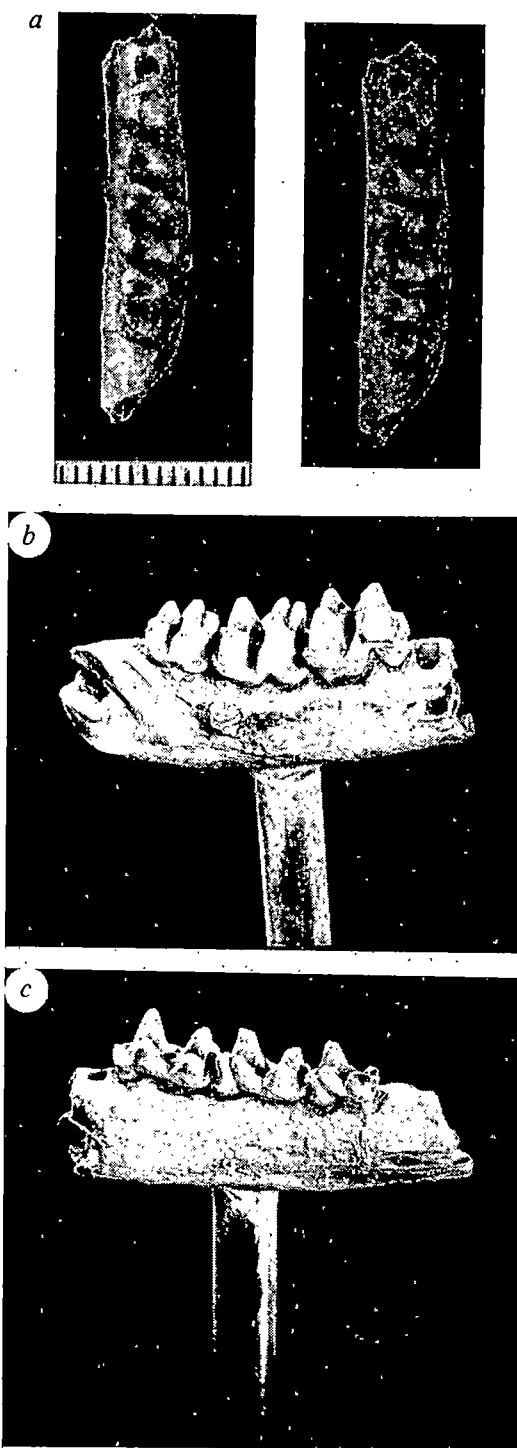


Fig. 1 The holotype of *Steropodon galmani* n. gen. and sp. in occlusal view (a), buccal view (b) and lingual view (c).

hypoconulids, presence of a distinctive distal metacristid and molars that exhibit essentially transverse shearing crests.

Description: While a more detailed description is in preparation, the above diagnosis, the comments made below and the illustrations (Figs 1, 2) will suffice for present purposes.

A posterior, elongate, shallow horizontal depression on the lingual side of the dentary extends from the posterior broken edge of the dentary forward to the level of the posterior root of M₃; this may represent a groove for the meckelian cartilage or it may be the result of pre-depositional breakage that seems to have affected other areas of the posterior part of the jaw fragment.

We conclude that *S. galmani* is a monotreme for the following reasons. Its antero-posteriorly very compressed trigonid,

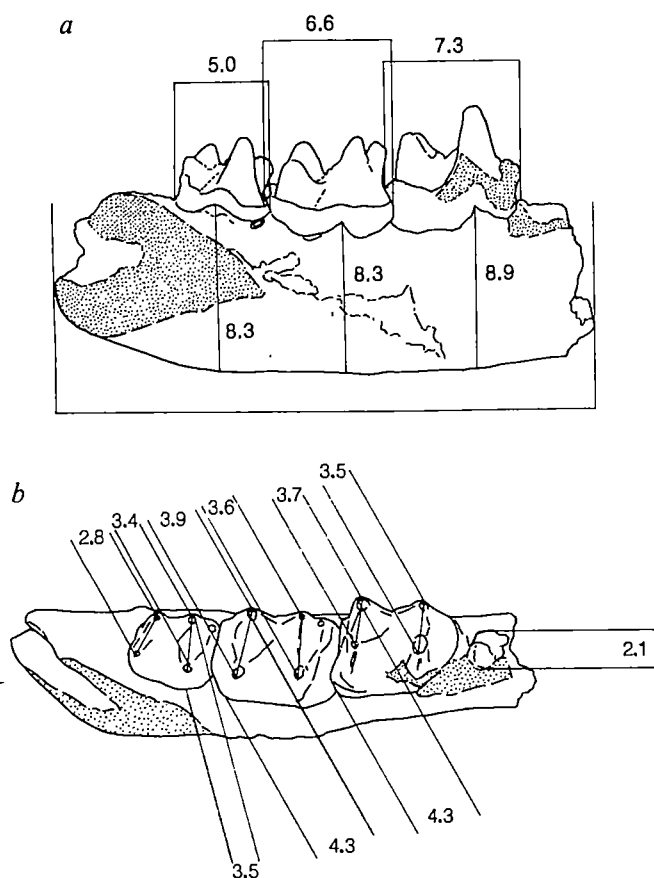


Fig. 2 Measurements (in mm) of the holotype in buccal view (a) and occlusal view (b).

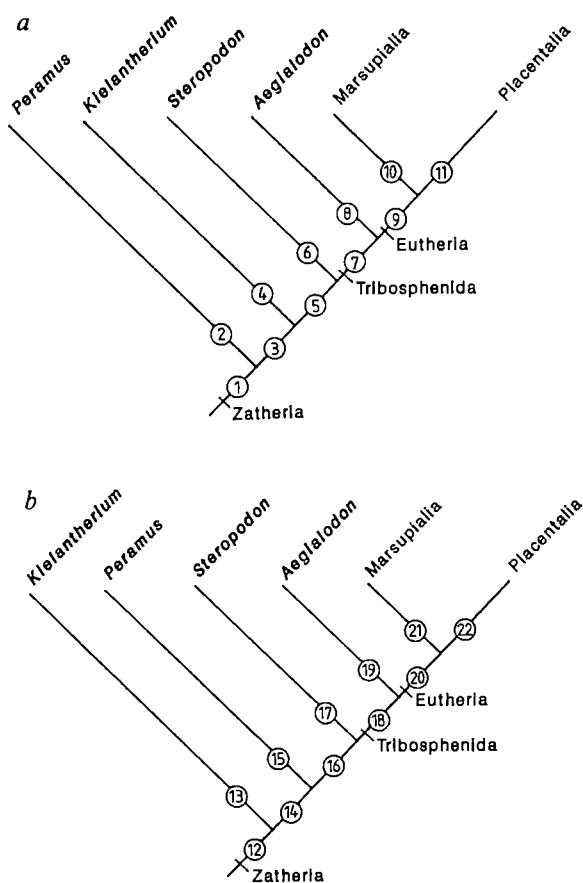


Fig. 3 The most-supported cladograms relating *Steropodon galmani*, the oldest and most plesiomorphic monotreme, to other groups of zatherian mammals. The possible relationships of monotremes to multituberculates are noted in the text. Cladogram a presumes that *Peramus tenuirostris* has four adult molars; b presumes that it has three. Potential synapomorphies are as follows: (1) reduction to four adult molars, incipiently basined talonids, stylocones reduced, anterior cingula of lower molars reduced, three talonid cusps; (2) lingually open talonid basins, premolariform first adult molar; (3) enclosed talonid basins with well-developed entocristids; (4) reduction of the entoconids as discrete cusps along the entocristids; (5) loss of postdentary bones from the lower jaw. ? loss of the meckelian groove, wide talonid basins, enlarged entoconids; (6) loss of one molar, paraconid lost from first adult molar, anterior cingula enlarged on lower molars, very wide talonid basins, hypertrophied entoconids and hypoconids, extensive pre-entocristids, loss of hypoconulids, transverse prevallid and postvallid shear; (7) protocone developed; (8) marked reduction of anterior cingula on the lower molars; (9) very wide talonid basins, enlarged protocones, transverse prevallid and postvallid shear, reduction of the postmetacristids; (10) loss of deciduous premolars, enlarged stylar cusp C, enlarged metacones, approximated entoconids and hypoconulids; (11) loss of one adult molar, reduction of stylar cusps, molariform dP4; (12) reduction to four molars, incipiently basined talonids, two talonid cusps (the hypoconid and hypoconulid), stylocones reduced, anterior cingula of lower molars reduced; (13) basined talonids with extensive pre-entocristids; (14) loss of one adult molar, entoconids developed, ? loss of meckelian groove; (15) submolariform posterior premolar, small lingual talonid basins; (16) loss of postdentary bones from the lower jaw, completely enclosed and wide talonid basins, well-developed pre-entocristids; (17) loss of the paraconid from the anterior adult molar, enlarged anterior cingula on the lower molars, hypertrophied entoconids and hypoconulids, loss of hypoconulids, transverse prevallid and postvallid shear; (18) protocone developed, pre-entocristids reduced; (19) marked reduction of anterior cingula on the lower molars; (20) same as (9); (21) same as (10) plus supernumerary molars (normally there are four adult molars in marsupials as well as a deciduous first molar); and (22) reduction of stylar cusps, molariform dP4.

absence of a paraconid on M_1 , high transverse loph-like trigonid and talonid crests, very large talonid, lack of a hypoconulid, and prominent anterior, posterior and buccal cingula are distinctive features that in combination also occur only in the isolated lower molars of the middle Miocene monotreme *Obdurodon insignis*^{3,13}. Further, the dental formula of this Lightning Ridge mammal includes three molars, the number evidently also present in *Ornithorhynchus anatinus*, the only living monotreme to retain even vestigial teeth. However, this number of molars may be a symplesiomorphic feature (see below).

The Lightning Ridge monotreme is significant in that it has relevance for interpretations of relationships among the major groups of mammals. At present, the phylogenetic position of monotremes is in considerable doubt. There are three main hypotheses in vogue: (1) monotremes (possibly with multituberculates, haramiyids and triconodonts) are prototherian mammals and as such the sister-group of the therian mammals; (2) monotremes and multituberculates are monophyletic but this group is one of three trichotomous mammal branches, the other two being the triconodonts and the therian mammals; (3) monotremes (and possibly multituberculates) are therian mammals, as opposed to the prototherian triconodonts (with docodonts) and, within Theria, they form either the sister-group of Zatheria (peramurids plus tribosphenids; refs 4, 14, 15; but see also ref. 16) or the sister-group of the Tribosphenida (Aegialodontidae plus Eutheria). We consider that the Lightning Ridge monotreme provides strongest support for the third hypothesis. Features of *S. galmani* that suggest monophyly with tribosphenid therian mammals include the apparent loss of postdentary bones, the wide and enclosed talonid basins, the enlarged entoconids and the well-developed pre-entocristids (Fig. 3).

Because of uncertainty about the molar number of the peramurid *Peramus tenuirostris*¹⁶⁻¹⁸, we are unable to decide whether peramurids or the newly described *Kielantherium*

*gobiensis*¹⁶ represents the plesiomorphic sister-group of monotremes plus tribosphenids. While Dashzeveg and Kielan-Jaworowska¹⁶ suggest that *K. gobiensis* is a tribosphenid similar to *Aegialodon dawsoni*, the presence in the latter but not the former of three well-developed talonid cusps and a wide talonid basin seems to us to be sufficient reason to question the inclusion of both forms within the same family. Pending the results of studies of monotreme occlusion, we suggest that the structure of monotremes (as evidenced by the albeit autapomorphically specialized *S. galmani*) represents an evolutionary stage between these two zatherians (Fig. 3).

The fact that distinctive monotremes were present in Australia in the early Cretaceous indicates not only the antiquity of this group but also that by this time at least some elements of Australia's fauna were exhibiting regional endemism of the sort that characterizes much of the continent's known Cenozoic faunas⁹. However, the paucity of knowledge about the early Cretaceous mammals of other continents means that we cannot exclude the possibility that monotremes also occurred on other continents in the Cretaceous.

One additional curious aspect of *S. galmani* is its large size. Not only is it the largest mammal known from the early Cretaceous, it may be one of the two largest Mesozoic mammals known, the other being the badger-sized late Cretaceous stagodontid *Didelphodon vorax*¹⁹. Overlap in body size between Mesozoic mammals and dinosaurs has not previously been recorded²⁰. The Lightning Ridge local fauna, however, includes some very small hypsilophodontid dinosaurs which were very little if at all larger than *S. galmani*.

Finally, the Lightning Ridge monotreme is heraldic because it comes from a continent that so far has failed to produce any pre-Miocene terrestrial mammals². As such, it represents a long-overdue reassurance that Australia did have an early Tertiary mammal record and that perseverance in the search will be worthwhile.

We thank Esso Australia, the State Government of New South Wales and the Director and Trustees of the Australian Museum for raising or providing funds necessary to acquire the Galman collection for the Australian Museum. We also acknowledge the contribution made by David and Alan Galman who bought and assembled a unique collection of opalized fossils from Lightning Ridge, including the *S. galmani* dentary. Michael Plane and Brian Senior helped in interpreting the geological setting of the specimen. Robert Jones helped with preparation of the specimen and John Fields, Alex Ritchie and Jenny Taylor produced the photographs. Colleagues who also contributed thoughts about the significance of the specimen include Richard Tedford, Michael Plane, Michael Woodburne, Neville Pledge, Thomas Rich, Suzanne Hand, Lyndal Dawson and Kenneth Aplin. Zophia Kielan-Jaworowska provided a cast of the important *K. gobiensis*.

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Kinship, reciprocity and synergism in the evolution of social behaviour

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There are two ways to model the genetic evolution of social behaviour. Population genetic models using personal fitness¹⁻⁹ may be exact and of wide applicability, but they are often complex and assume very different forms for different kinds of social behaviour. The alternative, inclusive fitness models¹⁰⁻¹², achieves simplicity and clarity by attributing all fitness effects of a behaviour to an expanded fitness of the actor. For example, Hamilton's rule states that an altruistic behaviour will be favoured when $-c + rb > 0$, where c is the fitness cost to the altruist, b is the benefit to its partner, and r is their relatedness. But inclusive fitness results are often inexact for interactions between kin¹⁻⁵, and they do not address phenomena such as reciprocity¹³⁻¹⁵ and synergistic effects^{7,8,16} that may either be confounded with kinship or operate in its absence. Here I develop a model the results of which may be expressed in terms of either personal or inclusive fitness, and which combines the advantages of both; it is general, exact, simple and empirically useful. Hamilton's rule is shown to hold for reciprocity as well as kin selection. It fails because of synergistic effects, but this failure can be corrected through the use of coefficients of synergism, which are analogous to the coefficient of relatedness.

For simplicity, assume that all members of a diploid population interact in pairs, that each may or may not have an opportunity to aid its partner and that the genetic component of altruism is due to two alleles at a single locus, with no overdominance. Table 1 defines the random variables used in the model. Each is defined over the whole population. The phenotypic

Table 1 Random variables used in the model

- G = an individual's frequency of the altruism allele ($0, \frac{1}{2}$ or 1)
 P = an individual's phenotypic value (1 when altruistic, 0 when not)
 W = total fitness
 W_0 = fitness if aid is neither given nor received
 C = fitness cost if individual were to be altruistic (that is, if $P = 1$)
 B = fitness benefit if partner were to be altruistic (that is, if $P' = 1$)
 D = deviation from additivity of C and B if both were altruistic (that is, if $PP' = 1$)
 G', P', B', D' = an individual's partner's values of G, P, B, D

values (P, P') are defined as either 1 or 0 , where, in contrast to inclusive fitness models, the latter case applies to all individuals who did not perform the altruism, including those who had no opportunity to do so. Fitness components are also defined for all individuals, for example, C is defined, even for a non-altruist, as the cost it would incur if it were altruistic. Finally, assume that each fitness component is distributed independently of G, G', P and P' .

An individual's fitness, W , can be written as

$$W = W_0 - CP + BP' + DPP' \quad (1)$$

where multiplication by the phenotypic values ensures that the fitness components are counted only when the appropriate behaviours are performed. Assuming a fair meiosis, Price's selection equation¹⁷ shows that $\Delta \bar{G} = \text{Cov}(G, W) / \bar{W}$, so the

altruism allele is favoured ($\Delta \bar{G}$ positive) when $\text{Cov}(G, W) > 0$. Substituting for W from equation (1) and splitting into four terms yields

$$\text{Cov}(G, W_0) - \text{Cov}(G, CP) + \text{Cov}(G, BP') + \text{Cov}(G, DPP') > 0 \quad (2)$$

Since W_0 , C , B and D are distributed independently of G , G' , P and P' , the first term is zero and the remainder may be rewritten as $-\bar{c} \cdot \text{Cov}(G, P) + \bar{b} \cdot \text{Cov}(G, P') + \bar{d} \cdot \text{Cov}(G, PP') > 0$, where \bar{c} , \bar{b} and \bar{d} are the sample means of C , B and D .

The mean fitness effects have now been extracted from the covariances that embody the population structure. Dividing through by $\text{Cov}(G, P)$ yields:

$$-\bar{c} + \bar{b} \cdot \frac{\text{Cov}(G, P')}{\text{Cov}(G, P)} + \bar{d} \cdot \frac{\text{Cov}(G, PP')}{\text{Cov}(G, P)} > 0 \quad (3)$$

This is still a personal fitness formulation since all fitness effects are assigned to the individual affected rather than to the one responsible for the effect. An inclusive fitness form is obtained by rewriting condition (2) as $\text{Cov}(G, W_0) + \text{Cov}(G, CP) + \text{Cov}(G', B'P) + \text{Cov}(G', D'P'P) > 0$. The same set of interactions are scored for genotype, phenotype and fitness effect. In the third and fourth terms we have simply switched the arbitrary labels designating when an individual is considered as self and when as someone else's partner. Mathematically this amounts to reordering the terms within the covariance, leaving the total unchanged. Proceeding as before now yields

$$-\bar{c}' + \bar{b}' \cdot \frac{\text{Cov}(G', P)}{\text{Cov}(G, P)} + \bar{d}' \cdot \frac{\text{Cov}(G', P'P)}{\text{Cov}(G, P)} > 0 \quad (4)$$

We now have equivalent results from the viewpoints of personal fitness (condition 3) and inclusive fitness (condition 4). When costs and benefits are additive, $\bar{d} = \bar{d}' = 0$, and the third terms disappear. What remains is Hamilton's rule, using covariance measures of relatedness previously proposed by Orlove and Wood¹⁸ (condition 3) and by Michod and Hamilton¹⁹ (condition 4). Here, however, these measures are taken over all individuals in the population, not just those with an opportunity to perform altruism, and this minor change makes the two measures equivalent.

These relatedness coefficients actually measure more than pedigree connections. The derivation is unchanged for altruism expressed in a conditional manner: be altruistic to an untested partner; otherwise, behave towards the partner as he behaved towards you. This reciprocal altruism strategy can be selected for the same reason as altruism towards kin: covariance between the performance of the behaviour and the recipient's frequency of the altruism allele. With reciprocal altruism a positive covariance arises when a partner's prior behaviour serves as a reliable indicator of possession of the reciprocity genes.

The third terms of conditions (3) and (4) correct Hamilton's rule for non-additivity. They consist of the product of the average deviation effect, \bar{d} , and a population structure coefficient. I will call these s coefficients, or coefficients of synergism, because they depend on the joint behaviour of both partners (PP'). In every other respect they are the same as the r coefficients measuring relatedness and reciprocity; both depend on a covariance between the behaviour(s) causing a fitness effect and the genes of the individual receiving it. These s coefficients are positive, even when interactants are not relatives (though they approach zero for very low P and P'), so that the sign of \bar{d} alone determines the direction of error in Hamilton's rule. Since relatedness is not required, the third term is best viewed as being due to something other than kin selection: a form of trait group selection⁹ or, to use a more evocative term, synergistic selection¹⁶. In this view, Hamilton's rule is correct for pure kin selection (and, as shown above, reciprocal altruism); its failure is caused by a distinct process.

A variety of biological phenomena can be represented by the D term²⁰. Several examples are given below. In each, D represents some combined effect of two altruists, acting either

sequentially or simultaneously, beyond their summed effects if they acted alone.

For behaviours in which costs and benefits take the form of successively altered survival probabilities, such as warning calls, a multiplicative fitness model is appropriate²¹. If we let the base fitness be unity, the fitness of an individual receiving both cost and benefit is not $1 - C + B$, but $(1 - C)(1 + B)$ or $1 - C + B - BC$, so that $D = -BC$. The negative deviation from additivity means that altruism will be harder to select than predicted by Hamilton's rule, as other models have shown^{4,5}. In effect, benefits are squandered when the recipient dies because of its own altruism.

A distasteful insect with warning coloration runs an increased risk of being seen and eaten by a predator. This cost may be compensated if the bad taste causes the predator to avoid its victim's neighbours²². A general avoidance of neighbours, regardless of their coloration, would be represented by B , and this could select for warning coloration if neighbours tend to be related. But any additional avoidance of only those neighbours with warning coloration must be represented by a positive D because this gain applies only to those who play the same strategy as the altruist.

Male lions may cooperate in taking over or retaining a pride²³. In searching for a partner, a cooperative lion may incur some cost, C , whether or not the potential partner actually cooperates. The fitness gain, however, applies only if cooperation does ensue ($PP' = 1$), and is therefore represented not by B , but by a synergistic D . For both lion cooperation and warning coloration, the positive D means Hamilton's rule underestimates the ease with which these traits are selected. In fact, if the altruistic trait can reach non-zero levels by drift, the s coefficient will be positive even in the absence of relatedness, so that a positive D could sometimes select for altruism by itself.

The model is quite general. It is not restricted to altruistic behaviours, since B and C may be assigned negative values. Additional synergistic effects can be incorporated by adding terms to the fitness decomposition in equation (1). For example, if three altruists ($P = P' = P'' = 1$) cause some additional effect, E , beyond that caused by two, the added term would be $EPP'P''$. The starting point of the model, Price's equation, holds for any mating system and is easily adjusted for haplodiploidy. It is also valid for any linear function of any number of alleles at any number of loci¹⁷. Thus, G and G' may be interpreted as the breeding value for the altruistic behaviour. Since this value can be estimated through breeding experiments²⁴, the model should be useful in empirical studies.

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Plasticity of functional epithelial polarity

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The fundamental characteristics that allow vectorial transport across an epithelial cell are the differential sorting and insertion of transport proteins either in the apical or the basolateral plasma membrane, and the preferential association of endocytosis and exocytosis with one or the other pole of the cell. Asymmetrical cellular structure and function, being manifestations of terminal differentiation, might be expected to be predetermined and invariant. Here we show that the polarity of transepithelial H^+ transport, endocytosis and exocytosis in kidney can be reversed by environmental stimuli. The HCO_3^- -secreting cell in the cortical collecting tubule is found to be an intercalated cell possessing a Cl^-/HCO_3^- exchanger in the apical membrane and proton pumps in endocytic vesicles that fuse with the basolateral membrane; the H^+ -secreting cell in the medullary collecting tubule has these transport functions on the opposite membranes. Further, the HCO_3^- -secreting cell can be induced to change its functional polarity to that of the H^+ -secreting cell by acid-loading the animal.

We identified a subset of cells in isolated cortical collecting tubules using the mitochondrial accumulation of the fluorescent cation 3,3'-dipentylloxycarbocyanine (Di-O-C₅(3)) (Fig. 1a). These mitochondrion-rich cells are enriched in carbonic anhydrase and transport protons^{1,2}; that is, they are the intercalated cells. We stained cortical collecting tubules with 6-carboxyfluorescein diacetate; this is a permeant nonfluorescent precursor of the pH-sensitive dye 6-carboxyfluorescein which is released by cytoplasmic esterases such as carbonic anhydrase, so these cells will preferentially accumulate the dye (Fig. 1b). There were 100–150 intercalated cells per mm of tubule, accounting for about 25% of all the cells in this segment (L. M. Satlin and G.J.S., unpublished observations). The cytoplasmic pH (pH_i) of the intercalated cell, measured by excitation ratio microspectrofluorometry^{2,4}, was on average 0.4 pH units higher than that of the principal cell. When collecting tubules were perfused with fluorescent macromolecules, only 5–10% of the intercalated cells in the cortical tubule, but most of those in the medullary segment, endocytosed the fluorophores (Fig. 1c–e)³. Since the medullary collecting tubule secretes H^+ whereas the cortical collecting tubule secretes HCO_3^- (Table 1), it is likely that the intercalated cell showing luminal endocytosis is the H^+ -secreting cell, while that lacking it secretes HCO_3^- .

To test this directly, we removed chloride from the basolateral solution and measured pH_i in individual intercalated cells simultaneously identified as those showing or not showing endocytosis by the uptake of rhodamine-bovine serum albumin (BSA) (see Table 1 legend). Removal of basolateral chloride stimulated HCO_3^- secretion in the cortical collecting tubule and inhibited H^+ secretion (HCO_3^- absorption) in the medullary collecting tubule (Table 1). It also increased the cell pH by 0.2 ± 0.1 units in both cortical and medullary intercalated cells that had endocytosed rhodamine-BSA from the lumen (Table 1). The increased cellular pH and decreased H^+ secretion in medullary collecting tubules indicate that the lumenally endocytosing cell has a basolateral Cl^-/HCO_3^- exchanger and is the H^+ -secreting cell. In the cells that did not endocytose rhodamine-BSA, removal of basolateral Cl^- decreased cell pH by 0.4 ± 0.1 pH units (Table 1). As this manoeuvre stimulated HCO_3^- secretion, the reduction in pH_i indicates that this cell is the HCO_3^- -secreting cell, with a Cl^-/HCO_3^- exchanger located in the luminal membrane.

Table 1 Net HCO_3^- transport ($J_{HCO_3^-}$) and cell pH (pH_i) in individually identified intercalated cells of isolated perfused cortical and medullary collecting tubules

	$J_{HCO_3^-}$ ($\mu\text{mol min}^{-1}$ mm^{-1})	Luminal endocytosis	pH_i No luminal endocytosis
Cortical collecting tubule			
Control	-3.3 ± 1.6 $n = 13$	7.23 ± 0.06 $n = 7$	7.34 ± 0.09 $n = 7$
Cl^- -free bath	$-12.0 \pm 2.3^\dagger$	$7.40 \pm 0.09^*$	$6.96 \pm 0.13^*$
Medullary collecting tubule			
Control	9.1 ± 1.6 $n = 5$	7.14 ± 0.07 $n = 5$	—
Cl^- -free bath	$2.1 \pm 1.6^*$	$7.33 \pm 0.04^\dagger$	—

One to four cells were studied in each perfused tubule to provide a mean for each animal. Net fluid absorption in four cortical collecting tubules perfused and bathed in identical solutions was negligible (control = 0.023 ± 0.024 ; Cl^- -free bath = 0.022 ± 0.027 $\text{nl min}^{-1} \text{mm}^{-1}$, $P > 0.5$). The tubules were perfused at $1-2$ $\text{nl min}^{-1} \text{mm}^{-1}$. Bicarbonate concentration in nl samples was measured by microcalorimetry¹⁰ and net bicarbonate transport was calculated from the product of the difference in HCO_3^- concentration between perfused and collected fluids and the flow rate, and normalized to mm of tubular length. All experiments were done at 38°C and used an artificial solution (290 ± 1 mosmol per kg) containing (in mM): NaCl (114), $NaHCO_3$ (25), K_2HPO_4 (2.5), $CaCl_2$ (2.0), $MgSO_4$ (1.2), glucose (5.5), L-alanine (6.0), Na-lactate (4.0) and citrate (1.0); it was gassed with 95% $O_2/5\%$ CO_2 to yield a pH of 7.4. At least three collections of tubular fluid were obtained for measurement of total CO_2 . Plus values denote bicarbonate absorption (H^+ secretion) while negative values denote bicarbonate secretion. Cell pH was measured in individually identified cells using 20–30 μM 6-carboxyfluorescein diacetate in the bath to load the cells for 5 min with 6-carboxyfluorescein. Simultaneously, the lumen was perfused with the same artificial solution containing rhodamine-BSA as a fluorescent macromolecule at $0.5-1$ mg ml^{-1} . The cells that endocytosed the fluorescent macromolecule, considered to be H^+ -secreting cells, appeared red under the rhodamine filter cassette (Fig. 1d, e) and bright green under the fluorescein filter block (Fig. 1b). Those cells which did not endocytose rhodamine from the lumen appeared bright green under the fluorescein filter cassette and showed no red staining with the rhodamine filters. Each cell was identified as rhodamine-positive or -negative and centred in the microscope photometer aperture. The filter cassette was then changed to fluorescein, the single cell was excited at 450 and 490 nm, and the emission was recorded at 535 nm in a Farrand microspectrofluorometer via the photometer attached to the camera port of the inverted microscope. As there were so few cells that did not endocytose rhodamine-BSA in the medullary collecting duct, we did not study such cells in that segment. Because the pH sensitivity of 6-carboxyfluorescein tends to saturate at pH values > 7.3 , these isolated tubules were studied at a pCO_2 of 50–55 mm Hg. This mild respiratory acidosis slightly decreased the cell pH. At the end of each experiment, the tubule was exposed to 100 μM of the electrogenic proton ionophore CCCP (carbonyl cyanide *p*-chlorophenyl hydrazone) in 150 mM KCl plus 10 mM phosphate buffer. This combination depolarized the cell and clamped the pH of the cytoplasm to that of the bath. The pH of the bath was compared with that measured fluorimetrically in 5–15 cells of the clamped tubules. The recorded pH values were obtained from a calibration curve over a pH range of 5.5–7.5 in the same calibration buffer. The fluorescence intensity of the 6-carboxyfluorescein-containing extracellular calibration solution was chosen to be approximately the same as the intensity of the cells studied *in vitro*, $\sim 1-2$ μM . The average pH difference between the fluorimetric cellular pH and the pH of the bath as measured by a microelectrode was 0.02 ± 0.03 units ($n = 10$ tubules) and was not a significant difference.

* $P < 0.05$; † $P < 0.01$ (Cl^- -free bath versus control). Mean \pm s.e., n = number of animals.

Secretion of HCO_3^- by a lumenally located Cl^-/HCO_3^- exchanger implies that a proton-translocating H^+ -ATPase exists at the basolateral membrane. Such proton pumps are packaged in endocytic vesicles, as we have shown in the H^+ -secreting cells²⁻⁴. To demonstrate basolateral endocytosis into acid vesicles, fluoresceinated dextran (relative molecular mass 70,000) was added to the basolateral medium of collagenase-treated cortical collecting tubules. Some, but not all, intercalated cells endocytosed the macromolecules into vesicles (Fig. 1f) that maintained an intravesicular pH of 6.0 ± 0.2 ($n = 14$ tubules, with 5–6 cells measured per tubule), similar to that found previously in cells endocytosing fluorescein-dextran from the lumen^{2,3}. By measuring total cellular fluorescence in 6 tubules (4–5 cells studied per tubule), we found that increasing ambient pCO_2 induced the exocytotic release of $40 \pm 3\%$ of the basolaterally endocytosed fluorescein-dextran (or the pH-insensitive dye Lucifer yellow), similar to results found for the lumenally endocytosing cell³.

To confirm the presence of basolateral endocytosis, we exposed collagenase-treated cortical collecting tubules to horse-

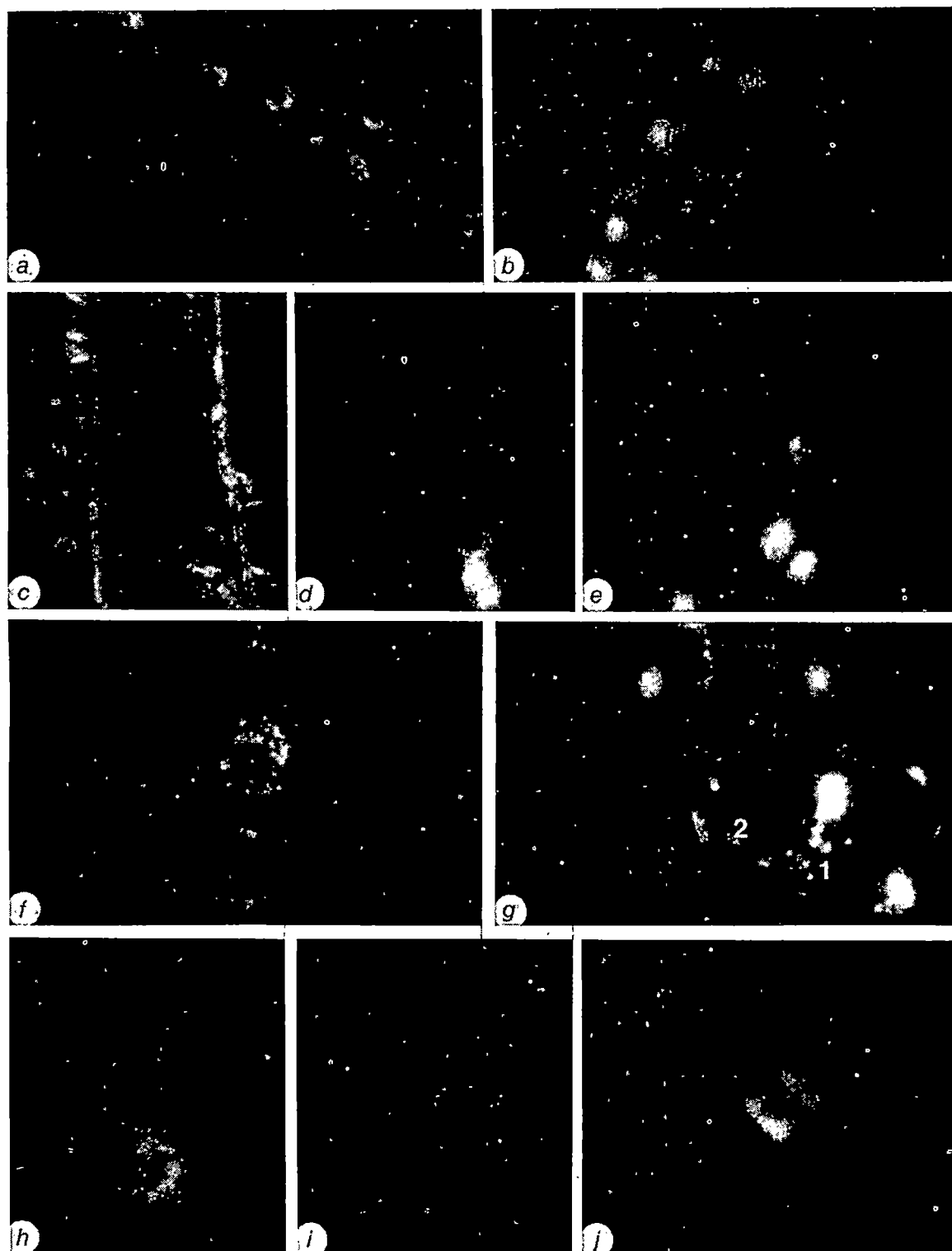


Fig. 1 *a*, Isolated cortical collecting tubule incubated for 5 min in 50 nM Di-O-C₃(3), a fluorescent cation which accumulates in mitochondria. At this low concentration of the dye the reticular, perinuclear pattern is seen only in some cells, the mitochondrion-rich (intercalated) cells $\times 200$. *b*, Isolated cortical collecting tubule incubated for 5 min in 20 μ M 6-carboxy fluorescein diacetate. Bright fluorescence is seen in the mitochondrion-rich cells. These cells are enriched in carbonic anhydrase, a potent esterase, and thus preferentially take up this dye. Further, as these cells are more alkaline, the dye will appear brighter than in the other cells. $\times 200$. *c, d*, Cortical collecting tubules perfused for 10 min with rhodamine-BSA (1 mg ml⁻¹) and then washed free of luminal fluorophore. In *c*, using Hoffman modulation contrast optics, note the presence of intercalated cells with a characteristic bulging surface at the top right and bottom right. *d*, The same field using epifluorescence optics. Only the bottom right cells have internalized the fluorophore. The pattern of fluorescence is diffuse, with negative nuclear staining. Because of the enrichment of the cells with vesicles and consequent light scattering, it is difficult to resolve individual vesicles. $\times 300$. *e*, Fluorescence micrograph of a medullary collecting tubule perfused in a similar manner to that of *d*. Endocytosis of the marker is seen in many more cells than in the cortical collecting tubule in *d*. $\times 200$. *f*, Isolated perfused cortical collecting tubule incubated for 20 min with 3 mg ml⁻¹ Lucifer yellow in the basolateral bathing medium. After extensive washing the tubule was mounted between two coverslips and photographed. A punctate uptake of this small non-permanent dye is seen in some cells. $\times 500$. *g*, Isolated cortical collecting tubule incubated for 2 min in 10 μ M acridine orange. This weak base is concentrated in acid vesicles and its emission spectrum undergoes a redshift at high concentration. The cell numbered 1 shows a concentration of sub-apical vesicles with no basolateral acid vesicles, suggesting that it is the H⁺-secreting cell. Cell 2 (probably a HCO₃⁻-secreting cell) has many sub-apical and basolateral vesicles. Addition of 5 μ M nigericin, a K⁺H⁺ exchange ionophore, abolished the orange staining in the vesicles, indicating that the dye accumulated in these vesicles because of the presence of a pH gradient across their membranes. $\times 500$. *h, i*, A cortical collecting tubule perfused at 38 °C for 10 min with rhodamine-BSA (1 mg ml⁻¹), washed free of the dye, cooled to 9 °C for 3 min and perfused with fluorescein-conjugated peanut lectin (0.5 mg ml⁻¹). After washout of fluorescein lectin, the tubule was mounted between two coverslips and photographed at $\times 500$. *h*, Note two patterns of fluorescence, one appearing diffuse with a negative nuclear stain while the other shows sharper edges characteristic of surface binding. *i*, The same field observed with rhodamine filters. Note endocytosis of the macromolecules only in the cell that shows a diffuse pattern and negative nuclear stain in *h*. *j*, Collecting tubule isolated from the ampulla of the newborn rabbit kidney, fixed with 2% glutaraldehyde in 0.1 M cacodylate buffer (pH 7.4) containing 10 μ g ml⁻¹ Hoechst 33258, the fluorescent chromatin-binding stain. After 1 h of exposure the segment was mounted between two coverslips. Note that the chromosomes are condensed in the polar positions of anaphase. $\times 500$. Tubules isolated as described in Table 1.

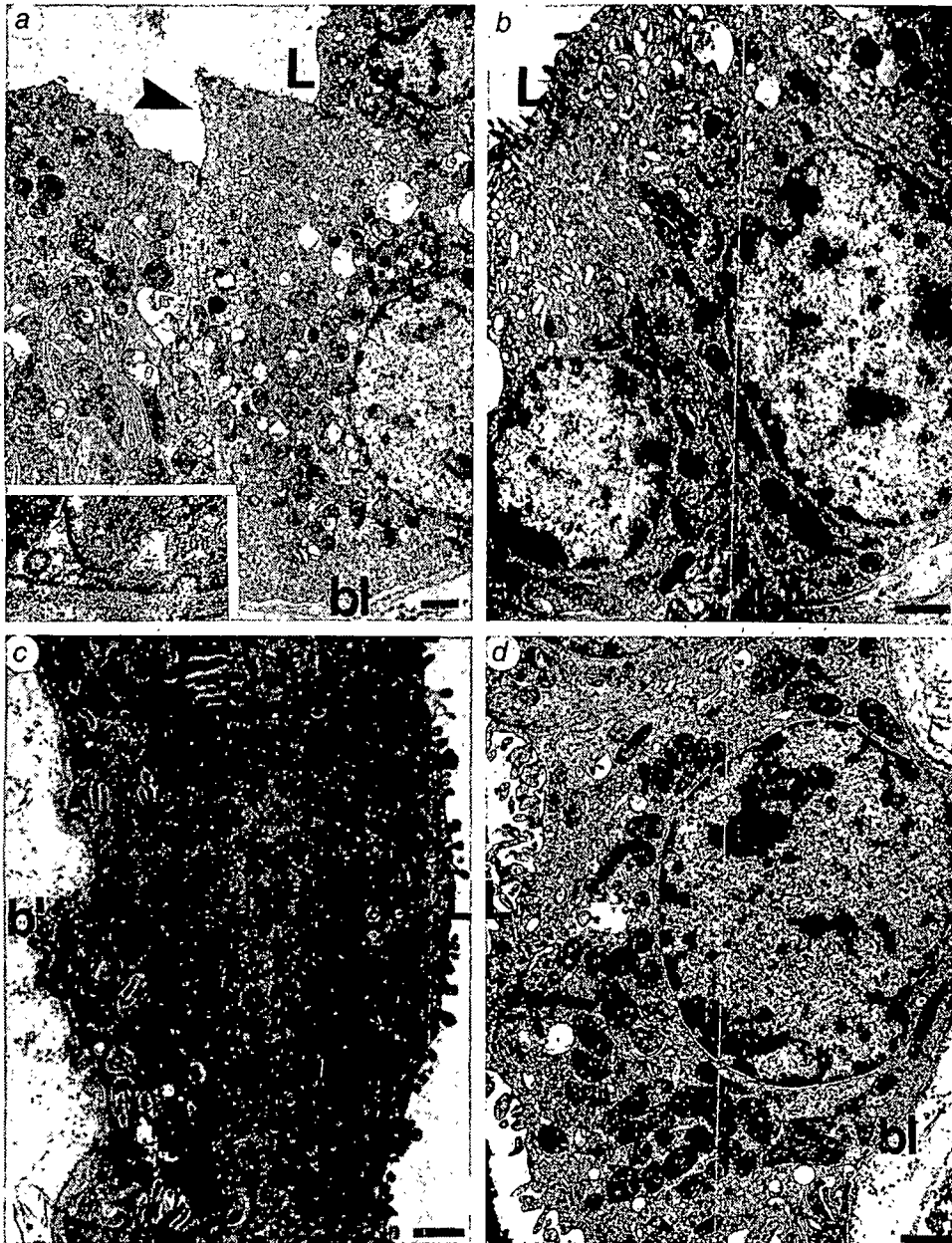


Fig. 2 Electron microscopy of cortical collecting tubules in intact kidneys (*a, b*) and in isolated tubules incubated for 10 min in 2% horseradish peroxidase (type VI, Sigma) (*c, d*). Tubules were fixed with 4% glutaraldehyde and post-fixed with 1% OsO_4 . They were then reacted with the diaminobenzidine/glucose/glucose oxidase system of Itoh *et al.*¹¹ for 30 min after pretreatment with 0.1% CoCl_2 . Two types of intercalated (mitochondria-rich) cells are seen. In *a* and *c* apical vesicles are surmounted by a dense cytoskeletal meshwork (*a*, arrowhead) and coated endocytic vesicles and omega figures are found along the basolateral membrane (*a*, inset). In *b* and *d* the apical cytoplasm is less dense and fusions between the apical vesicles and luminal membrane can be seen. Perfusion of the basolateral surface with horseradish peroxidase in isolated tubules for 10 min (*c, d*) showed considerable basolateral endocytosis in the mitochondria-rich cells having apical cytoskeletal caps (*c*, arrowheads) but no such uptake was found in the other apical vesicle-rich cell (*d*). Longer periods of exposure to horseradish peroxidase resulted in uptake of the marker in all cells. Hence, cell *c* has a higher rate of basolateral endocytosis than the other cells. bl, Basolateral; L, luminal. Scale bar, 1 μm ; inset in *a*, $\times 48,000$.

radish peroxidase from the basolateral side only. Electron microscopy revealed at least two types of intercalated cells⁵ (Fig. 2). Only one type showed basolateral uptake of horseradish peroxidase (compare Fig. 2*c* and *d*). The cells that endocytosed horseradish peroxidase contained large numbers of sub-apical vesicles (Fig. 2*a, c*), separated from the plasmalemma by a dense 'mat' of fibrillar cytoskeletal matrix (Fig. 2*a*). Also apparent were many endo-exocytic (omega) figures and coated vesicles at the basolateral membrane (Fig. 2*a*, inset). In the other type of intercalated cell these omega figures and coated vesicles occurred predominantly at the apical membrane (Fig. 2*b, d*). Such coated vesicles (probably clathrin-coated vesicles) are known to contain the H^+ -ATPase⁶⁻⁸. Both sub-apical and basolateral vesicles were acidic, as shown by the accumulation of the weak base acridine orange (Fig. 1*g*). Cell 2 in Fig. 1*g* is probably the HCO_3^- -secreting cell containing apical and basolateral acid vesicles; cell 1 is probably an H^+ -secreting cell containing a large sub-apical concentration of vesicles. The principal cells did not contain many acid vesicles.

When animals were given a chronic acid load, the transport properties of cortical collecting tubules removed 24 h later and perfused *in vitro* at pH 7.4 were converted from HCO_3^- secretion

to H^+ secretion (Table 2). This was accompanied by a large increase in the number of H^+ -secreting cells (that is, cells showing luminal endocytosis) (Table 2). To test whether the increased H^+ secretion was due to a conversion of the polarity of the HCO_3^- -secreting cell or to a proliferation in the H^+ -secreting cell, we counted both H^+ - and HCO_3^- -secreting cells. Intercalated cells were identified by their ability to bind peanut lectin⁹. When fluorescent peanut lectin was perfused into the lumen of the cortical collecting tubule, two staining patterns emerged—bright surface staining with sharp edges (Fig. 1*h*), crescentic in perfused tubules, and more diffuse staining reminiscent of the endocytic pattern, with the nucleus observed as an unstained region (Fig. 1*h*). When the tubule was perfused with macromolecules labelled by a different fluorophore, only the cells with diffuse lectin staining endocytosed the macromolecules (compare Fig. 1*h* and *i*). Chronic acid loading did not increase the total number of intercalated cells per mm of tubule (control 129 ± 5 , $n = 15$ tubules; acid-loaded 137 ± 6 , $n = 10$ tubules, $P > 0.5$). Rather, the increase in the number of H^+ -secreting cells was accompanied by an equivalent decrease in the number of HCO_3^- -secreting cells (Table 2). Further, if the 10-fold increase in the number of H^+ -secreting cells seen after

Table 2 Effects of acid loading on isolated perfused cortical collecting tubules in rabbits

	$J_{\text{HCO}_3^-}$ ($\mu\text{mol min}^{-1}$ mm^{-1})	Potential difference (mV)	H^+ - secreting cells	HCO_3^- - secreting cells
Control	-1.6 ± 0.5 (4)	-13 ± 3 (4)	7 ± 2 (15)	122 ± 4 (15)
Acid-loaded	5.4 ± 0.8 (7)†	-5 ± 2 (7)*	74 ± 10 (10)†	63 ± 11 (10)†

Tubules were from control rabbits or from rabbits given NH_4Cl (10–20 mequiv per kg per day by gavage) for 5 ± 1 days. Cell counts for luminal endocytosis (10 control and 9 acid-loaded rabbits) were obtained by perfusing each tubule for 10 min at 38°C with $0.5\text{--}1\text{ mg ml}^{-1}$ fluorescein-dextran³ dissolved in a bicarbonate-free solution (290 ± 1 mosmol per kg) containing (in mM): NaCl (139), K_2HPO_4 (2.5), CaCl_2 (2.0), MgSO_4 (1.2), glucose (5.5), L-alanine (6.0), Na-lactate (4.0) and $\text{Na}_3\text{-citrate}$ (1.0); pH was 7.4. The chamber was then cooled to $9\text{--}12^\circ\text{C}$ for 5 min, after which rhodamine-peanut agglutinin was perfused in the lumen for 2–3 min. The solution was washed out with cold fluorophore-free solution and the tubule was warmed to room temperature for study by epifluorescence microscopy. At least three $250\text{-}\mu\text{m}$ segments of each cortical collecting tubule were examined for numbers of green, fluorescein-positive or luminally endocytosing cells, and for red or lectin-binding cells. Despite the cooling of the specimen chamber, it was not always possible to rule out endocytosis of peanut lectin by the acid-secreting cells, which could lead to an overestimate of the number of the HCO_3^- -secreting cells. The total number of cells per mm was determined by counting nuclei in duplicate at $\times 1,000$ over three to six $50\text{-}\mu\text{m}$ lengths of each cortical collecting tubule that was fixed according to Fig. 1j legend. The total number of cells per mm of tubule was similar in both groups: control, 561 ± 8 ; acid-loaded, 543 ± 11 ($P > 0.2$). Bicarbonate transport was measured in a second perfused cortical collecting duct isolated from the same kidney slice. Transepithelial voltage (PD) was measured using the luminal pipette as an electrode and the bath solution as a reference³. The three to five measurements of potential difference were made at the time of each collection of tubular fluid for determination of bicarbonate concentration. Both luminal and bathing solutions were identical (290 ± 1 mosmol per kg) and contained (in mM): NaCl (114), NaHCO_3 (25), K_2HPO_4 (2.5), CaCl_2 (2.0), MgSO_4 (1.2), glucose (5.5), L-alanine (6.0), Na-lactate (4.0) and $\text{Na}_3\text{-citrate}$ (1.0); they were gassed with 95% $\text{O}_2/5\%$ CO_2 to yield a pH of 7.4. Bicarbonate transport was measured by microcalorimetry as described in Table 1 legend. Ammonium chloride administration caused a chronic acidosis in most rabbits, the mean total CO_2 in the plasma being 22 ± 2 mM compared with 28 ± 1 mM in controls. Moreover, the urine pH was lower: 6.1 ± 0.4 in ammonium chloride-loaded rabbits and 8.5 ± 1.4 in control animals. Note that the administration of ammonium chloride did not necessarily cause a chronic acidosis in all animals, because the kidneys were markedly stimulated to increase acid excretion. Some animals showed normal levels of total CO_2 , yet the tubules from the animals absorbed bicarbonate at high rates and the number of cells that could endocytose fluorescent dextran from the lumen was markedly higher than in control animals.

* $P < 0.05$; † $P < 0.01$ (acid-loaded versus control). Mean \pm s.e. (number of tubules).

a few days of acid loading was caused by cellular proliferation, mitotic figures should be apparent. We saw such figures (using the dye Hoechst 33258) only in the ampulla of the collecting duct of newborn rabbits (Fig. 1j). There was only one mitotic figure in 27 tubules from 5 control animals (15,150 cells) and none in 70 tubules from 10 acid-loaded animals (38,170 cells). To examine the possibility that mitosis occurred early in acid loading, we studied eight tubules (4,280 cells) removed from animals after 1 day of acid loading, but found no mitotic figures even though the number of H^+ -secreting cells had increased three to fivefold. Thus, the observed increase in the number of H^+ -secreting cells is caused by a reversal of the functional polarity of the HCO_3^- -secreting cell, and this reversal accounts for the conversion of the tubule from HCO_3^- secretion to H^+ secretion.

The conversion of HCO_3^- -secreting cells to acid-secreting cells requires not only relocation or activation/inactivation of the transporting elements (the proton ATPase, the parallel Cl^- channel and the $\text{Cl}^-/\text{HCO}_3^-$ exchanger) at the appropriate pole, but also remodelling of the cytoplasm to allow exocytosis and endocytosis at the apical pole of the cell and to inhibit them at the basolateral domain (Fig. 2). Such responses must also be stable for at least 24 h.

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Upstream sequences modulate the internal promoter of the human 7SL RNA gene

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The human genome is rich in sequences which are structurally related to the 7SL RNA component of the signal recognition particle¹. The 7SL DNA sequence family consists of four 7SL genes², 500 7SL pseudogenes² (which are truncated at one or both ends of the 7SL sequence) and 500,000 *Alu* sequences^{3–5}. Both 7SL genes² and *Alu* elements^{6–8} are transcribed by RNA polymerase III, and we show here that the internal 7SL promoter lies within the *Alu*-like part of the 7SL gene. Why then does RNA polymerase III transcribe the few 7SL genes so efficiently, while transcripts from the far more abundant *Alu* elements are not readily detectable^{9–11}? We find that a human 7SL gene and a synthetic *Alu* sequence derived from it are expressed 50–100-fold more efficiently *in vitro* than either a representative *Alu* element or two 7SL pseudogenes. 5' Deletion and insertion mutants of the 7SL gene demonstrate that, in conjunction with the internal promoter, the first 37 nucleotides upstream from the transcription start site are essential for efficient and accurate initiation *in vitro*. We suggest that the genomic sequences upstream from most *Alu* elements and 7SL pseudogenes do not contain this element, and consequently that only a small subset of such sequences can be transcribed *in vivo*. This may help to explain the homogeneity of the *Alu* family within each mammalian genome, as well as the species-specific differences between mammalian *Alu* families.

To determine which sequences in the human 7SL gene are important for expression, we compared the *in vitro* transcription of one of the four human 7SL genes, 7L30.1 (ref. 2) (Fig. 1A, lane 30), two 3'-truncated 7SL pseudogenes, 7L28 and 7LEM1 (ref. 2) (lanes 28 and EM1), and a pBR322 derivative of the human 7SL cDNA clone p7L1 (ref. 12), called p7L1-bis (lane 1; see Fig. 1B for the structures of the clones). Although all the 7SL clones were transcribed, the wild-type 7SL gene was 50–100 times more efficient than the other templates. The gene-pseudogene fusions (lanes 30–28 and 28–30) show that the sequences required for efficient transcription lie predominantly to the 5' side of the *Sma*I site in the 7SL gene (Fig. 1B). Transcription of the 7SL cDNA clone p7L1-bis proves that the 7SL coding region contains an internal promoter.

We also compared the transcription of a cloned *Alu* element with that of the 7SL gene (Fig. 1C). For this analysis we chose the *Alu* sequence located upstream from the human γ -globin gene, which is transcribed *in vitro* to yield a major transcript of ~575 nucleotides⁶. Since full activity of the 7SL promoter² requires an unusually low concentration of Mg^{2+} , the *Alu* and 7SL gene templates were assayed over a range of Mg^{2+} concentrations. The optimum for the 7SL gene was 1 mM Mg^{2+} ,

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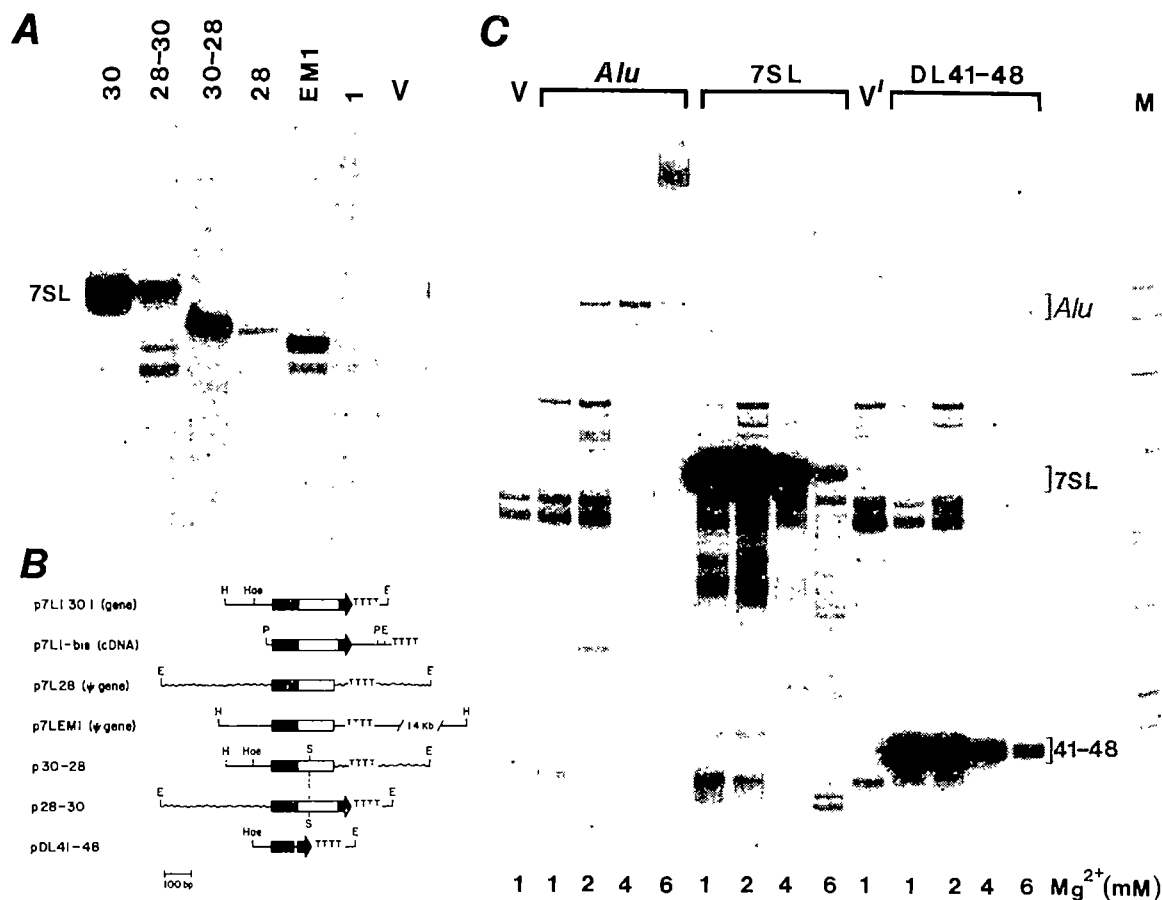


Fig. 1 **A**, The products of *in vitro* transcription of gene 7L30.1² (lane 30, 7SL RNA-300 nucleotides; nt), 3'-truncated pseudogenes 7L28 (lane 28, 7L28 RNA ~275 nt) and 7LEM1 (lane EM1, 7LEM1 RNA 265 nt)², 7L1-bis cDNA (lane 1, 7L1 RNA ~440 nt), recombinants 7L30-28 (lane 30-28) and 7L28-30 (lane 28-30) and pUC13 vector DNA (lane V). Transcription of 7SL templates in a HeLa cell nuclear extract³¹, fractionation and purification of the transcripts were carried out as described previously². To quantitate RNA synthesis, labelled RNA bands were detected by autoradiography, excised from the gel and Cherenkov radiation was counted. In different experiments the yield of ³²P radioactivity per band was: 7L30.1 RNA, 2.5–4 × 10⁵ c.p.m.; 7L28 RNA, 2–4 × 10⁵ c.p.m.; 7LEM1 RNA, 5–10 × 10⁵ c.p.m.; 7L1-bis RNA 2–4 × 10⁵ c.p.m.; 7L30-28 RNA, 1.5–2 × 10⁵ c.p.m.; 7L28-30 RNA 5–10 × 10⁵ c.p.m. As assayed by T1 RNAase fingerprinting, the major transcripts derived from all templates initiated at or near the normal start site² and terminated at a run of four or more T residues in the template DNA (see **B**; data not shown). **B**, Schematic representation of the 7SL clones used for *in vitro* transcription. p7L30.1² contains human 7SL gene 7L30.1 including the entire coding region plus ~180 base pairs (bp) of 5'-flanking and 30 bp of 3'-flanking DNA in pUC13. p7L28 and p7LEM1 are pUC13 plasmid subclones derived from 7SL pseudogenes 7L28 and 7LEM1². Both pseudogenes are colinear with the 7SL coding sequence up to position 235, but their genomic context is different. p7L1-bis contains a complete cDNA copy of human 7SL RNA from clone p7L1¹² inserted into pBR322 so that the T₃ oligonucleotide at vector position 4,324 is immediately 3' to the 7SL coding region. Plasmid subclones p7L30.1 and p7L28 were recombined using the unique *Sma*I site at position 143 in the 7SL coding region. The DNA fragment corresponding to the 5' half of the gene was ligated to the 3' fragment of the 7L28 pseudogene to create p7L30-28, and the 5' half of the pseudogene was ligated to the 3' half of the gene to create p7L28-30. Plasmid pDL41-48 is a recombinant derived from p7L30.1-5'-66 (see below) in which the nucleotides between positions 85 and 248 have been deleted and substituted with an 8-nt *Xho*I linker. The arrows represent the various 7SL DNAs and indicate the direction of transcription; the shaded areas represent the *Alu* sequence of 7SL DNA^{4,12}; H, *Hind*III; E, *Eco*RI; S, the *Sma* site used to recombine p7L30.1 and p7L28; P, *Pst*I; Hae, *Hae*III; TTTT, indicates the position of the transcription terminator³². **C**, Products of *in vitro* transcription of the 7SL gene 7L30.1 (lanes 7SL), clone pBS2⁶ (lanes *Alu*) which contains the *Alu* sequence upstream from the human γ -globin gene, and of clone pDL41-48 (lanes 41-48) as a function of varying Mg^{2+} concentration. The amount of exogenous Mg^{2+} added is indicated below each lane. V, pBR322 transcripts; V', pUC13 transcripts; M, end-labelled *Hpa*II DNA fragments of pBR322 as relative molecular mass markers. The yield of transcripts from clone pBS2 was comparable to that from pseudogene 7L28. The human genomic *Alu* clone pEB77 (ref. 33) and the monkey *Alu* clone p7.01 (ref. 34) gave a similar result.

while the *Alu* sequence was transcribed best at 4 mM Mg^{2+} . Even when the transcription activities of the two templates were compared at their respective Mg^{2+} optima, the 7SL gene was at least 100 times more active than the genomic *Alu* clone. A similar result was obtained with two additional genomic *Alu* sequences (see Fig. 1C legend). Taken together, these results suggest that either the 5'-flanking DNA or the central non-*Alu* portion of the 7SL coding region (the S sequence¹²) might be responsible for the high level of 7SL gene transcription.

To test whether sequences in the non-*Alu* portion of the 7SL coding region are required for efficient transcription, we deleted the S sequence from the 7SL gene. This synthetic *Alu* sequence (pDL41-48, Fig. 1B) produced the expected shorter transcript

of ~140 nucleotides (Fig. 1C, lanes DL41-48), was as active as the 7SL gene itself, and exhibited the same Mg^{2+} optimum. We conclude that the 5'-flanking sequence of gene 7L30.1 is likely to be responsible for its high expression, and that the internal control region must reside within the *Alu*-like parts of 7SL DNA because the S sequence is not required for transcription *in vitro*.

Next, we constructed a series of 7SL genes with progressive deletions of 5'-flanking DNA (Fig. 2A). Deletion of sequences downstream of nucleotide -37 had two effects on *in vitro* transcription (Fig. 2B). First, mutant 7SL genes with less than 37 base pairs of 5'-flanking sequence displayed a significant reduction in activity. For instance, the 5'-2 mutant, which contains only the 7SL coding sequence plus 30 nucleotides of 3'-flanking

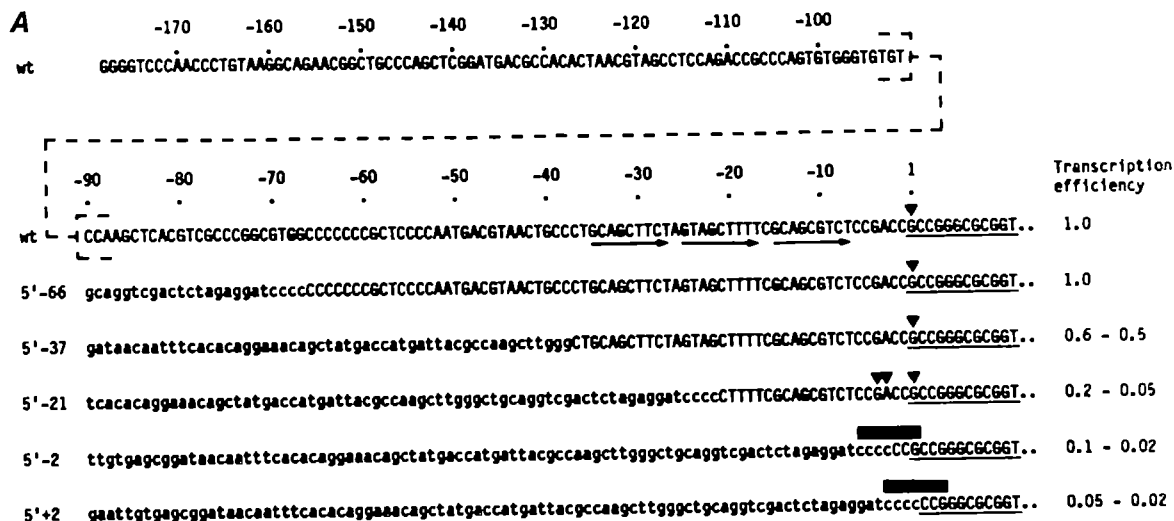
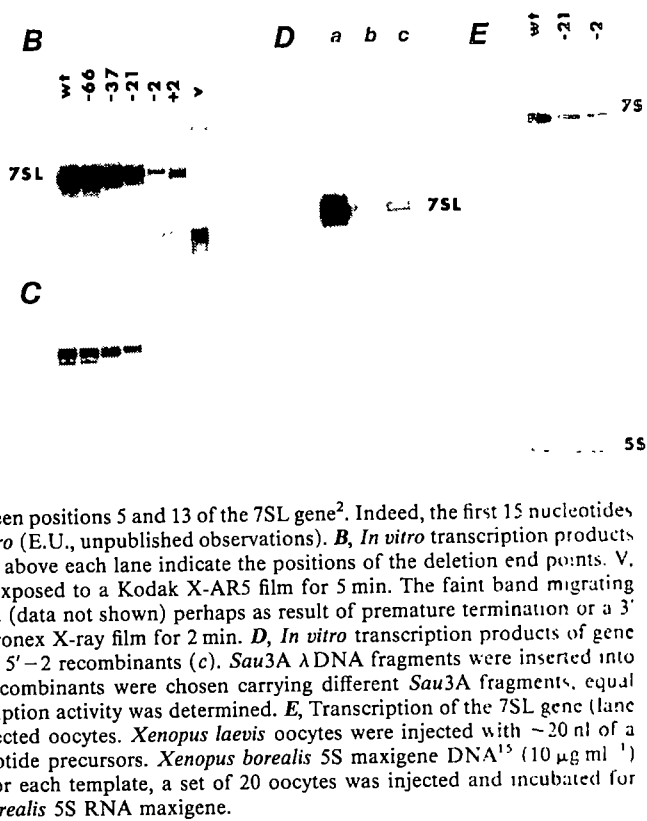


Fig. 2 A, Nucleotide sequences and transcription efficiencies of the 5' deletion derivatives of the 7SL gene 7L30.1 (wt). Progressive deletions of the 5'-flanking DNA of gene 7L30.1 were generated either by digestion with restriction enzymes or with *Bal31* nuclease. The 5' deletion derivatives are named according to the position of the deletion end point. Structures of the deleted templates were verified by DNA sequence analysis using the chemical degradation method³⁵. The 5'-37 recombinant was constructed by digestion of p7L30.1 with *Pst*I plus *Eco*RI, followed by insertion of the resulting fragment at the corresponding sites in pUC13 DNA. The other 5' deletions were inserted between the *Sma*I and *Eco*RI sites of the same plasmid vector. The beginning of the 7SL RNA coding sequence is underlined. Upper case letters: natural 5'-flanking DNA; lower case letters: pUC13 vector DNA. Arrowheads: transcription initiation sites mapped by T1 fingerprint and 5' end-group analysis. Solid bar: presumptive initiation sites; we did not determine the precise initiation sites of 5'-2 and 5'+2 RNAs, but their 5' ends mapped within the cluster of C residues abutting the 7SL coding sequence. Arrows below the wild-type sequence indicate a set of three 9-bp direct repeats. The transcription efficiencies represent both the highest and the lowest obtained using several different preparations of HeLa cell nuclear extract. Note that a sequence closely resembling the A block consensus sequence of transfer RNA promoters³⁶ is located between positions 5 and 13 of the 7SL gene². Indeed, the first 15 nucleotides upstream from the 7SL coding sequence are essential for expression *in vitro* (E.U., unpublished observations). **B**, *In vitro* transcription products of the 7SL gene 7L30.1 (wt) and of the 5' deletion derivatives. Numbers above each lane indicate the positions of the deletion end points. V, the products of transcription of pUC13 vector DNA. The wet gel was exposed to a Kodak X-AR5 film for 5 min. The faint band migrating faster than 7SL RNA is a 7SL transcript which is truncated at the 3' end (data not shown) perhaps as result of premature termination or a 3' exonuclease activity. **C**, As in **B** except that the gel was exposed to a Cronex X-ray film for 2 min. **D**, *In vitro* transcription products of gene 7L30.1 (a), the 5'-2 deletion mutant (b) and a pool of 13 independent 5'-2 recombinants (c). *Sau*3A λ DNA fragments were inserted into the *Bam*HI site at position -10 of the 5'-2 clone; 13 independent recombinants were chosen carrying different *Sau*3A fragments, equal amounts of the various DNAs were pooled, and their cumulative transcription activity was determined. **E**, Transcription of the 7SL gene (lane 30), the 5'-21 (lane 21) and the 5'-2 (lane 2) deletion mutants in injected oocytes. *Xenopus laevis* oocytes were injected with ~20 nl of a solution containing plasmid DNA (400 μ g ml⁻¹) and radioactive nucleotide precursors. *Xenopus borealis* 5S maxigene DNA¹⁵ (10 μ g ml⁻¹) was co-injected as an internal control for the efficiency of injection. For each template, a set of 20 oocytes was injected and incubated for 20-24 h at 20 °C. 5S indicates the products of transcription of the *X. borealis* 5S RNA maxigene.



DNA, was 10-50 times less active than the wild-type gene. The upstream vector sequences do not contain an inhibitor of transcription, because replacement of vector DNA by a random collection of bacteriophage λ DNA fragments had no effect (Fig. 2D). Second, a good proportion of the transcripts produced from these mutant 7SL genes were larger than 7SL RNA (Fig. 2C). As judged by T1 fingerprinting and 5'-end-group analysis, approximately 10% of the transcripts from deletion mutants 5'-21, 5'-2 and 5'+2 were initiated correctly, while the remainder had 5' ends mapping a few nucleotides upstream from the G residue at position 1 (see Fig. 2A; data not shown). The failure of these 7SL deletion mutants to accumulate significant levels of 7SL RNA must reflect a decrease in the efficiency of transcription, since pulse-chase experiments *in vitro* did not reveal any difference in the turnover rate of the 5'-2 RNA as compared with authentic 7SL RNA (data not shown).

To determine whether the efficiency of transcription depends on the distance between the 5'-flanking sequences and the 7SL coding region, we constructed a second set of mutant 7SL genes

with 9, 11 and 13 extra nucleotides inserted at position -6 of the 7L 5'-66 template (Fig. 3A). Relative to the parent template (Fig. 3B), these mutations caused a ~10-fold reduction in transcription activity, and led to aberrant initiation upstream, as assayed by primer extension (Fig. 3C). Further increases in the length of the linker DNA inserted at position -6 did not significantly change the pattern of initiation observed with mutant DL+13 (data not shown). We conclude that the sequences upstream from the 7SL gene, in conjunction with the internal control region, provide information for positioning the correct start site of transcription; moreover, optimal transcription requires that the distance of the upstream DNA from the internal promoter is not altered.

Finally, the 5'-flanking sequence of the 7SL gene increases the efficiency of transcription from the 7SL internal promoter *in vivo* as well as *in vitro*. After injection into the nucleus of *Xenopus* oocytes, the 5'-21 and 5'-2 mutant templates (Fig. 2E) were transcribed 5-10-fold less efficiently than the wild-type gene, even though this is a heterologous system. In addition, the 5'-21 and 5'-2 transcripts were longer than 7SL

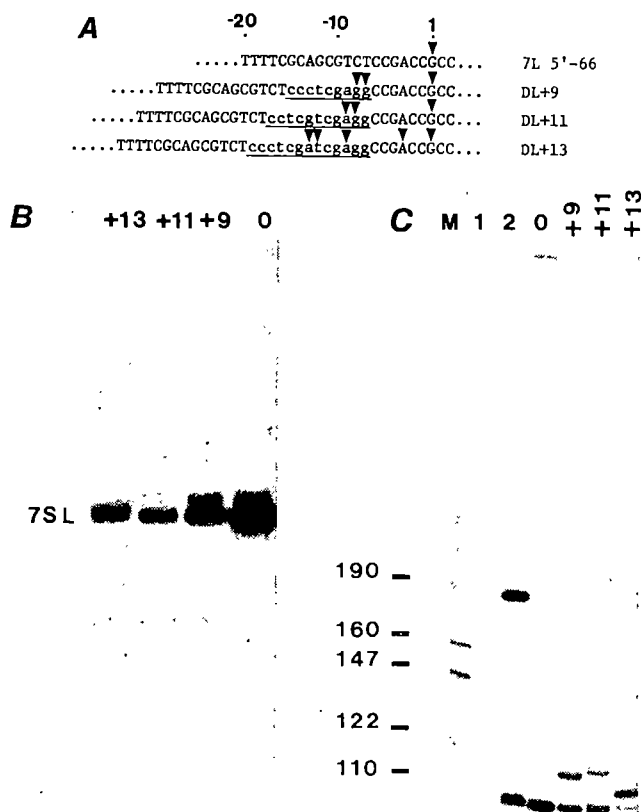


Fig. 3 **A**, Nucleotide sequences of mutant 7SL genes *DL+9*, *DL+11* and *DL+13* and position of the initiation sites mapped by primer extension (▼). The linker sequences are underlined. The *DL+9* recombinant was constructed by inserting an *XhoI* linker at position -6 of 7L 5'-66 DNA, after digestion³⁷ with DNase I in the presence of Mn²⁺. *DL+11* and *DL+13* were obtained by filling the ends of *XhoI*-digested *DL+9* DNA with Klenow polymerase and deoxynucleotide triphosphates. **B**, Products of *in vitro* transcription of 7SL mutants *DL+9* (lane +9), *DL+11* (lane +11) and *DL+13* (lane +13) and of 7L 5'-66 (lane 0). **C**, Primer extension analysis of 7SL RNA and of *DL+9*, *DL+11* and *DL+13* RNAs synthesized *in vitro*. The following RNAs were used as templates: lane 1, endogenous RNAs after micrococcal nuclease digestion; lane 2, endogenous RNAs before micrococcal nuclease digestion; lane 0, 7SL RNA synthesized from 7L 5'-66 DNA; lane +9, *DL+9* RNAs; lane +11, *DL+11* RNAs; lane +13, *DL+13* RNAs. M, end-labelled *HpaII* fragments of pBR322 DNA.

Methods. The 5' ends of the transcripts were mapped by primer extension using an oligonucleotide complementary to positions 96-110 of 7SL RNA. The endogenous RNAs of the transcription extract³¹ gave two major extension products of 110 and 190 nt (lane 2); the 110-nt cDNA corresponds to 7SL RNA, while the origin of the longer product is unknown. The extract was therefore digested with micrococcal nuclease before transcription (lane 1) to remove these endogenous RNAs. Micrococcal nuclease (5,000 U ml⁻¹) was added to HeLa cell nuclear extract containing 1 mM CaCl₂ and the digestion was carried out for 15 min at 30 °C. Nuclease digestion was stopped by addition of 2 mM EGTA; transcription reactions contained 500 µM of each of the four ribonucleotide triphosphates. Nucleic acids were purified by phenol extraction and, after ethanol precipitation, annealed to a 10-fold molar excess of the 5'-end-labelled synthetic oligonucleotide. Reverse transcription was carried out as described by Hernandez and Keller³⁸. cDNA products were fractionated through a 6% polyacrylamide gel containing 7 M urea.

RNA and identical in structure to the corresponding transcripts synthesized *in vitro*.

Our results have evolutionary implications. *Alu* elements are thought to transpose to new chromosomal sites using an RNA polymerase III transcript as an intermediate^{13,14}. The 5' end of the transposed *Alu* information is therefore defined by the initiation site for transcription of the parental *Alu* element, and each newly transposed *Alu* element acquires a new 5'-flanking sequence. Deletions and substitutions of the 5'-flanking sequences in a variety of genes transcribed by RNA polymerase III genes are known to affect the accuracy of initiation¹⁵⁻¹⁷ and to modulate either positively¹⁸⁻²² or negatively²³⁻²⁵ the efficiency of transcription. We have shown here that alterations in the 5'-flanking DNA of the human 7SL gene markedly decrease the efficiency of transcription and lead to the appearance of heterogeneous start sites. We speculate that the 5'-flanking sequences of most newly inserted *Alu* elements are unlikely to be compatible with maximal transcription and that only a small subset of mammalian *Alu* family members account for most *Alu* transcription *in vivo*. This subset of *Alu* elements ('founder elements') would produce most of the transcripts that serve as intermediates for transposition to new chromosomal sites. Mutations occurring in these founder elements when the copy number of *Alu* sequences was low at the time of the mammalian radiation, would lead to the observed species-specific differences between mammalian *Alu* sequences²⁶⁻²⁹, as the copy number of *Alu* sequences increased in each species³⁰.

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Tailoring the pH dependence of enzyme catalysis using protein engineering

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One of the goals of protein engineering is to tailor the pH dependence of enzyme catalysis to optimize activity in industrial processes. Chemical modification studies have shown that the pH dependence of catalysis by serine proteases alters with changes in overall surface charge¹, which suggests that a possible general method of modifying pH dependence is to alter the electrostatic environment of the active site by protein engineering and so change the pK_a values of ionic catalytic groups. Electrostatic effects are of considerable importance in enzyme catalysis and are thought to play a major part in stabilizing charged transition states²⁻⁵. However, it is extremely difficult to calculate electrostatic effects in proteins because of the microheterogeneity of the dielectric constant. In the present study we show how the change of just one surface charge which is 14–15 Å from the active site of subtilisin has a significant effect on the pH dependence of the enzyme and that the magnitude of the effect changes with ionic strength in a manner qualitatively consistent with electrostatic theory. Such experiments should provide the basis for refining theoretical calculations of electrostatic effects in catalysis.

The subtilisins are a family of extracellular serine proteases (also known as alkaline proteases) produced by species of bacilli prior to sporulation⁶. His 64 at the active site of the enzyme acts as a general base during catalysis, accepting a proton from the nucleophilic residue Ser 221 as it forms a bond with the substrate carbonyl carbon. The enzyme is active at alkaline pH when His 64 is unprotonated, and catalytic activity varies with pH following the ionization of this residue. The mutation we have chosen for our investigation of the magnitudes of electrostatic effects is Asp→Ser 99 in subtilisin from *Bacillus amyloliquefaciens* (BPN' or Novo). Crystallographic data show that this surface aspartate is some 14–15 Å from the imidazole of His 64 (ref. 7). (Note that because of a sequencing error, there is an alanine at position 99 in the early crystallographic models. We built in an aspartate at this position using molecular graphics. The derived distances have been substantiated by a new refined structure (R. Bott, personal communication)—the His 64 C γ -Asp C γ distance is 13 Å in the refined map, the same as in our modelling.) Asp 99 is not conserved in highly homologous subtilisins from other organisms: there is a serine at this position in the subtilisins from *Bacillus subtilis* DY⁸ and *Bacillus licheniformis*⁹ and threonine in subtilisins from *B. subtilis*¹⁰ and *Bacillus amylosacchariticus*¹¹. It is thus expected *a priori* that the mutation Asp→Ser 99 should not have a significant effect on either the structure or catalytic properties of the enzyme other than by electrostatic effects. Removal of the negative charge of Asp 99 should destabilize the low-pH, positively charged form of His 64 and so lower its pK_a from the normal value of 7 by an unknown amount.

To test the predictions and quantify the effects, we have cloned the structural gene encoding subtilisin BPN' from *B. amyloliquefaciens* in the vector pUB110 (refs 12, 13). The structure of pPT30, which harbours the subtilisin gene on a 3.4-kilobase (kb) *EcoRI* fragment, is shown in Fig. 1a; its construction will be described elsewhere. The DNA sequence of the structural gene was determined by dideoxy methods¹⁴ using a series of primers (J. Brannigan, unpublished data) and is identical to that published elsewhere^{15,16}. Wild-type subtilisin BPN' is expressed and secreted at high levels by *B. subtilis* DB104 transformed with pPT30 (Fig. 1b). The strain DB104, given by Dr R. Doi, is deficient in extracellular alkaline and neutral proteases¹⁷. Subtilisin(Asp→Ser 99), prepared by oligodeoxynucleotide

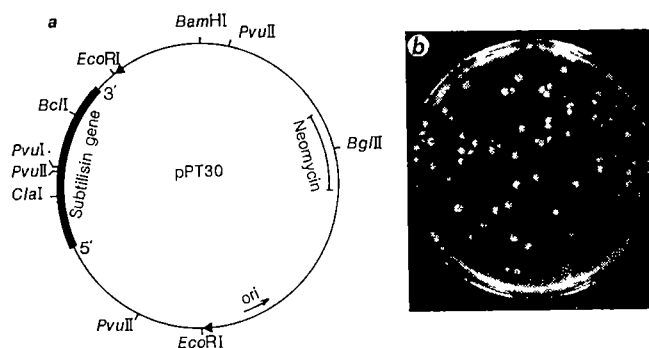


Fig. 1 a, Restriction map of the subtilisin expression vector pPT30. The subtilisin gene is contained within a 3.4-kb *EcoRI* fragment (solid block). Sequences derived from pUB110 (4.5 kb) are delineated by the arrowheads. b, Secreted subtilisin gives a characteristic clear halo around colonies of *B. subtilis* DB104 harbouring pPT30 after overnight selection on L-agar containing 1% skimmed milk plus 25 $\mu\text{g ml}^{-1}$ kanamycin.

Methods. For mutagenesis, the gene was recloned in M13mp9. M13aprWT was constructed such that the coding strand of the subtilisin gene was ligated to the (+) strand of the viral DNA. M13aprWT was propagated in *Escherichia coli* TG2, a *recA*⁻ derivative of JM101 (M. Biggin and T. Gibson, unpublished results) to overcome problems of DNA instability encountered when JM101 was the host. The synthetic primer (5'CGGAACCGCTAG-CACG3') contained a double mismatch with the wild-type gene (indicated by asterisks) and was designed to introduce an acceptable Ser codon at position 99. Oligodeoxynucleotide-directed mutagenesis was performed as described elsewhere^{20,21}, except that the mismatch repair-deficient strain BMH71-18mutL²² was the recipient in transformations. TG2 provided the lawn to isolate phage from the mutator background. Hybridization screening using the mutagenic primer as a probe showed that 20% of the plaques contained mutant phage. The mutation was confirmed by DNA sequencing after plaque purification. Subtilisin(Asp→Ser 99) was expressed in DB104 on transformation of competent cells²³ with a plasmid constructed by ligating the mutant gene isolated from replicative form DNA into pUB110 in the same orientation as pPT30. Wild-type and mutant enzymes were purified from 36-h culture supernatants grown in L-broth containing kanamycin (25 $\mu\text{g ml}^{-1}$) and glucose (0.2%). The supernatant was concentrated using a Pellicon membrane filter (PTGC00005, Millipore) before dialysis against 0.01 M phosphate buffer pH 6.2 and purification on a CM-52 cellulose column as described elsewhere (ref. 24).

mutagenesis (Fig. 1), is also expressed at similar high levels in DB104.

The activity of subtilisin(Asp→Ser 99) on the synthetic substrate succinyl-L-alanyl-L-alanyl-L-prolyl-L-phenylalanyl *p*-nitroanilide is similar to that of wild-type enzyme at 25 °C in 0.1 M Tris-HCl buffer, pH 8.6: k_{cat} (the catalytic rate constant) for the wild-type enzyme is 57 s⁻¹ and K_m (Michaelis constant) is 0.15 mM, while the corresponding values for the mutants enzyme are 45 s⁻¹ and 0.13 mM. The wild-type enzyme has identical activity to that produced from an independently cloned gene (ref. 15 and D. A. Estell and J. A. Wells, personal communication). The pK_a values of the active sites of the free enzymes were determined by kinetics from the pH dependence of k_{cat}/K_m (Fig. 2, Table 1). At ionic strength 0.1, His 64 in the wild-type enzyme has a pK_a of 7.17 ± 0.02 ; this is decreased to a value of 6.88 ± 0.03 in the mutant, a shift of 0.29 ± 0.04 units. k_{cat}/K_m is decreased by 20% on mutation. Thus, Asp 99 affects both the ionization constant of the active site and also the charged transition state of the reaction.

As a control, it is important to examine the effect of ionic strength on the electrostatic interactions. The presence of high concentrations of ions should mask electrostatic interactions. At high ionic strengths, the observed pK_a s should tend to their intrinsic values in the absence of surface charge. According to the classical Debye-Hückel theory and the Poisson-Boltzmann

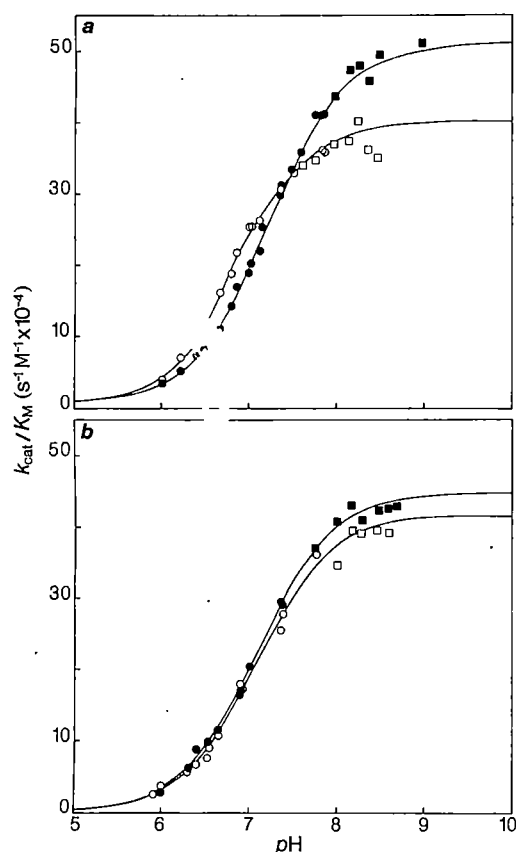


Fig. 2 pH dependence of k_{cat}/K_m for the hydrolysis of succinyl-L-alanyl-L-alanyl-L-prolyl-L-phenylalanyl *p*-nitroanilide by wild-type (solid symbols) and mutant Asp→Ser 99 (open symbols) subtilisin at 25 °C, ionic strength 0.1 (a) or 1.0 (b). Phosphate (circles) and Tris-HCl (squares) buffers of ionic strength 0.1 were used and the ionic strength adjusted by the addition of KCl. The reaction was initiated by adding 15 μl of enzyme (13 μM in 0.01 M phosphate buffer, pH 6.2) to 2 ml of buffered substrate (0.02 mM, $\ll K_m$) in a cuvette maintained at 25 °C in a Gilford 2600 spectrophotometer. The increase in absorbance at 412 nm on the release of *p*-nitroaniline was monitored and found to follow, with high accuracy, simple first-order kinetics. The value of k_{cat}/K_m at each pH value was determined from the first-order plots exactly as described for the hydrolysis of acetyl-L-tyrosine *p*-acetylanilide by chymotrypsin²⁵. The low concentration of substrate used in this method avoids product inhibition. The concentration of each enzyme was determined by active-site titration with *N*-trans-cinnamoyl imidazole²⁶. Wild-type enzyme was found to be 91% active and the mutant 96% active compared with concentrations measured by A_{280} ($E_{0.1\%} = 1.17$). Control experiments showed that the rate of reaction in Tris-HCl buffers was 15% lower than in phosphate buffers. The data for experiments in the presence of Tris-HCl have been corrected for this, but these data were not used in the analysis. The data for the experiments in phosphate buffer were fitted to a theoretical single ionization curve by nonlinear regression (R. J. Leatherbarrow, unpublished). The derived pK_a values are listed in Table 1.

equation, the electrostatic interaction between two ions separated by a distance r is reduced by a factor of $\exp(-r/r_D)$ where r_D , the Debye length, is inversely proportional to the square root of the ionic strength. At ionic strength 1.0, r_D is 3.1 Å and at ionic strength 0.1 it is 9.8 Å. Although the theory is only approximate at high ionic strength, electrostatic effects should be highly masked over a distance of 14–15 Å at ionic strength 1.0. Accordingly, we found (Table 1) that at this ionic strength, both the pK_a values of the active site and the catalytic activities of the wild-type and mutant enzymes converge ($pK_a = 7.11 \pm 0.02$ and 7.09 ± 0.02 ; $k_{\text{cat}}/K_m = (45 \pm 0.6) \times 10^4$ and $(42 \pm 1) \times 10^4 \text{ s}^{-1} \text{ M}^{-1}$ respectively). Thus, the mutation Asp→Ser 99 has essentially no effect on the catalytic properties of the enzyme when electrostatic effects are masked.

Table 1 The pH dependence of hydrolysis by wild-type and mutant subtilisin

Enzyme	pK_a of active site (from kinetics)	Limiting value of k_{cat}/K_m at high pH ($\text{s}^{-1} \text{ M}^{-1} \times 10^{-4}$)
Ionic strength 0.1		
Wild type	7.17 ± 0.02	48.9 ± 0.7
Asp→Ser 99	6.88 ± 0.03	40.8 ± 0.9
Ionic strength 1.0		
Wild type	7.11 ± 0.02	44.8 ± 0.6
Asp→Ser 99	7.09 ± 0.02	41.6 ± 0.9

The pK_a values were obtained from the pH dependence of k_{cat}/K_m on the hydrolysis of succinyl-L-alanyl-L-alanyl-L-prolyl-L-phenylalanyl *p*-nitroanilide at 25 °C. The pH dependence of k_{cat}/K_m gives the pK_a values of the free enzyme and free substrate. In this case, the only ionizing group on the substrate is the succinyl group and its pK_a is below the range of this study. Artefacts which often obscure the interpretation of the pH dependence of k_{cat} and K_m individually usually do not affect the pH dependence of k_{cat}/K_m (ref. 18). The pK_a of the wild-type enzyme is in excellent agreement with that derived from the hydrolysis of ester substrates in identical conditions (7.15 at ionic strength 0.1; ref. 19). Values are given \pm s.e.

We have shown that modification of a single charge in the vicinity of the active site of an enzyme can have a significant effect on the pH dependence of the catalytic reaction. Such effects should be larger for charges which are closer to ionic catalytic residues in the enzyme or closer to charges which develop in the transition states. Electrostatic effects will be more pronounced if multiple charges are engineered. It should, therefore, be possible to engineer large effects on both the pH dependence and catalytic rate constants by modifying the surface charge.

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Loss of genes on the short arm of chromosome 11 in bladder cancer

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Recent studies have shown that normal cellular sequences on chromosome 13 are lost during the development of retinoblastomas¹ and that sequences on chromosome 11 are similarly lost during the development of Wilms' kidney tumours²⁻⁵ and embryonal tumours⁶. Cells from these tumours have been found to contain either the paternal or maternal copies of loci on the affected chromosome, but not both. Thus, the somatic loss of heterozygosity for sequences on chromosome 13 or 11 is hypothesized to result in homozygosity for a recessive mutant allele on these chromosomes¹, and in this way the chromosomal loss may contribute to the development of these tumours. We sought to investigate whether similar losses of heterozygosity for chromosome 11 sequences occurred in a common adult tumour. We chose to analyse bladder cancers, since such cancers are common in the adult population and are derived from urogenital tissue, as are Wilms' tumours. We examined constitutional and tumour genotypes at loci on the short arm of chromosome 11 (11p) in 12 patients with transitional cell carcinomas. In five tumours, we observed the somatic loss of genes on 11p resulting in homozygosity or hemizygosity of the non-deleted alleles in the tumour cells. Our results show that the frequency of loss of 11p sequences in bladder cancer approaches that seen in Wilms' tumour (42% compared with 55%)⁷, and suggest that recessive genetic changes involving sequences on 11p may contribute to the development of bladder neoplasms.

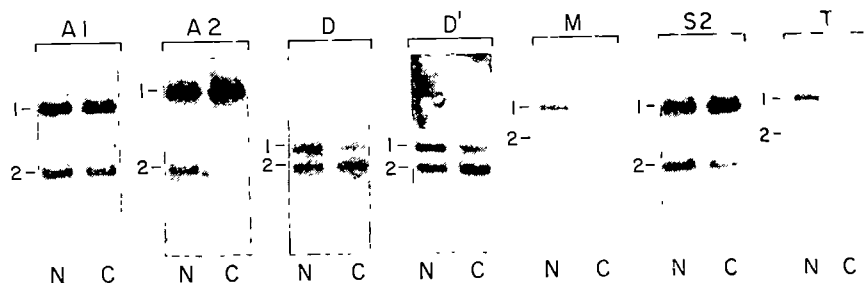
The patients studied were 50-85 years old and all had histologically confirmed transitional cell carcinoma (11 of the bladder, 1 of the ureter). All tumour tissues were obtained before any chemotherapy or radiotherapy, and the histological stage and grade of each patient's tumour is indicated in Table 1. DNA of high relative molecular mass was isolated from tumour and normal tissues (either normal bladder mucosa or peripheral leukocytes). To detect the somatic loss of chromosome 11p sequences in the tumours, we exploited DNA polymorphisms on chromosome 11. DNA polymorphisms are normal variations in DNA sequence that occur in the human population and may be used to distinguish the maternal and paternal copies of a gene. The variations can be detected by Southern blotting of

the patient's DNA using a cloned copy of the gene of interest as a probe. Comparison of DNA from normal and tumour tissue can reveal instances where either the paternal or maternal copy of a gene was lost during tumorigenesis.

Two genes on 11p that display DNA polymorphisms are *c-Ha-ras-1* and the insulin gene. *c-Ha-ras-1*, the human homologue of the transforming gene of the Harvey murine sarcoma virus, has been localized⁸ near the distal end of 11p. It displays a length polymorphism due to variations in the number of reiterations of a small tandemly repeated sequence located approximately 1.5 kilobases (kb) 3' to its coding region⁹. Similarly, the insulin gene has been assigned¹⁰ to 11p and it contains an insertion of variable length located approximately 1 kb 5' from the transcriptional start site of the gene¹¹. Figure 1 shows representative Southern blots of DNA from normal and tumour tissues probed with *c-Ha-ras-1* or insulin. Analysis of normal bladder DNA from patient A1 revealed two *c-Ha-ras-1* alleles (denoted 1 and 2 in Fig. 2). No loss of either allele was detected in tumour cells from this patient. Normal tissue from patient A2 was also polymorphic for the *c-Ha-ras-1* gene. However, in this patient's tumour tissue allele 2 was absent (see Fig. 1, lanes A2). Patient D was not heterozygous at the *c-Ha-ras-1* locus, but did display polymorphism at the insulin locus. His normal tissue revealed two insulin alleles (1 and 2 in Fig. 1, lanes D). DNA from his tumour tissue, however, showed significant loss of the hybridization signal from allele 1. Normal tissue from patients M, S2 and T was heterozygous at *c-Ha-ras-1*; in their tumour tissues, these patients had a marked reduction in the intensity of alleles 1, 2 and 1, respectively (see Fig. 1). All 12 patients were heterozygous at either the *c-Ha-ras-1* or the insulin locus. The six patients not shown in Fig. 1 exhibited no loss of 11p alleles in their tumour tissues (see Table 1). There was no apparent correlation between tumour stage or histological grade and the loss of 11p alleles.

To determine whether the losses of 11p alleles in tumour tissue were associated with duplications of the retained alleles, as was previously demonstrated in Wilms' tumours²⁻⁵ and embryonal tumours⁶, we quantified the hybridization signals seen in Fig. 1. At least one Southern blot filter from each of the patients was rehybridized with one or more DNA probes derived from sequences present on chromosomes other than 11. Autoradiographs of these blots were scanned with a densitometer and analysed to determine the ratios of hybridization of restriction fragments obtained from the normal and tumour DNA. In Fig. 2, it can be seen that the hybridization signal of the remaining 11p alleles in patients A2 and D is of approximately double intensity in the tumour cells, and is unchanged in patients M, S2 and T. Thus, the losses of 11p alleles in tumour cells of patients A2 and D were associated

Fig. 1 Representative Southern hybridizations of 11p gene probes to normal tissue (N) and transitional cell carcinoma (C) DNA. Patient designations are shown above the blots, and the numbers shown on the left indicate the polymorphic alleles (numbers correspond to the text and Table 1). Blots from patients A1, A2, M, S2 and T are *MspI* digests of DNA hybridized to the *c-Ha-ras-1* gene probe. DNA from patient D (and sample D') was digested with *HinfI* and hybridized to the insulin gene probe. Note the preservation of heterozygosity at the *c-Ha-ras-1* locus in tumour DNA of patient A1, while tumour DNA from patients A2, D, M, S2 and T reveal loss or marked reduction in the intensity of one allele. In addition, note the increased intensity of the retained allele in patients A2 and D. D' represents DNA extracted from frozen sections of patient D as described in the text.



from frozen sections of patient D as described in the text.

Methods. DNA of high relative molecular mass was prepared from bladder tumour biopsies and either normal bladder mucosa (patients A1, A2, D, M) or peripheral leukocytes (patients S2, T). The tissues were minced and digested overnight in 1% SDS and proteinase K (0.5 µg ml⁻¹) at 48 °C, and DNA was extracted with phenol and chloroform as described elsewhere¹². DNA concentrations were determined by a diphenylamine assay²¹. Approximately 1 µg of DNA was digested with each restriction enzyme, and the resulting DNA fragments were separated by electrophoresis through agarose gels and transferred to nitrocellulose filters^{22,23}. Gene fragments used as probes were: (1) a 1.0-kb *MspI* *c-Ha-ras-1* genomic fragment containing the polymorphic 3' flanking region²⁴; and (2) a 1.3-kb *PvuII* fragment from the recombinant plasmid hINS 5', containing the polymorphic sequences 5' to the insulin gene²⁵. The gene probe fragments were separated by electrophoresis and oligolabelled directly in agarose to a specific activity of >10⁹ d.p.m. µg⁻¹ (refs 26, 27). Southern hybridization²², post-hybridization washing²³ and autoradiography²⁹ were performed as described previously.

Table 1 Chromosomal alleles in normal tissue (N) or tumour tissue (C) in 12 transitional cell carcinomas

Patient	Stage + grade	1 ATIII	2 CPSI	3 DOSL C9	11 Ha-ras	11 Ins	12 $\alpha 1(II)$	13 p7F12	13 p9DII	13 p9A7	14 pAW101	15 DOSL C3	17 GH	18 DOSL C6	20 DOSL C2
A1 N	T2t3	2/2	3/3	1/1	1/2	1/2	1/1	1/2	1/2	1/1	1/2	1/1	2/2	—	1/2
C		2/2	3/3	1/1	1/2	1/2	1/1	1/2	1/2	1/1	1/2	1/1	2/2	—	1/2/2
A2 N	T3t2	1/2	2/3	1/1	1/2	1/2	1/1	1/2	1/2	1/2	1/2	1/1	1/2	2/2	1/2
C		1/2	2/	1/1	1/1	2/2	1/1	1/2	1/2	1/2	1/2	1/1	1/2	2/2	1/2
B N	T0t1	—	—	1/1	1/2	—	—	2/2	1/1	1/2	—	1/2	1/2	—	1/2
C		—	—	1/1	1/2	—	—	2/2	1/1	1/2	—	1/2	1/2	—	1/2
D N	T3t3	1/2	1/3	—	2/2	1/2	1/1	2/2	1/2	2/2	1/1	1/1	2/2	2/2	1/1
C		1/2	/3	—	2/2	2/2	1/1	2/2	1/2	2/2	1/1	1/1	2/2	2/2	1/1/1
G1 N	T0t2	—	—	1/1	1/2	—	—	1/1	2/2	2/2	—	2/2	2/2	—	1/2
C		—	—	1/1	1/2	—	—	1/1	2/2	2/2	—	2/2	2/2	—	1/2
G2 N	T3t3	1/2	2/3	1/1	1/2	1/1	1/1	2/2	2/2	2/2	1/1	2/2	1/2	2/2	1/1
C		1/2	2/3	1/1	1/2	1/1	1/1	2/2	2/2	2/2	1/1	2/2	1/2	2/2	1/1
M N	T3t3	1/1	3/3	1/1	1/2	—	1/1	—	1/1	1/2	1/2	—	1/2	—	1/1
C		1/1	3/3	1/1	/2	—	1/1	—	1/1/1	1/1/2	1/	—	1/2	—	1/1
P N	T0t2	1/1	2/3	1/1	1/2	1/2	1/1	1/2	1/1	2/2	1/2	1/2	2/2	2/2	1/1
C		1/1	2/3	1/1	1/2	1/2	1/1	1/2	1/1	2/2	1/2	1/2/2	2/2	2/2	1/1
S1 N	T0t1	—	—	—	1/1	1/2	—	1/2	1/2	1/1	—	1/2	1/2	—	1/2
C		—	—	—	1/1	1/2	—	1/2	1/2	1/1	—	1/2	1/2	—	1/2
S2 N	T1t3	1/1	3/3	1/1	1/2	1/2	1/2	1/1	1/2	1/2	1/1	1/2	1/2	2/2	1/2
C		1/1	3/3	1/1	1/	1/	1/2	1/1	1/2	1/2	1/1	1/2	1/2	2/2	1/2
T N	T0t2	2/2	1/3	1/1	1/2	1/2	1/2	1/1	1/2	2/2	1/2	1/2	1/2	1/2	1/2
C		2/2	1/3	1/1	/2	/2	1/2	1/1/1	1/2/2	2/2/2	1/2	1/2	1/2	1/2	1/2
W N	T0t2	—	—	2/2	2/2	1/2	—	1/1	1/1	2/2	—	1/2	2/2	—	1/1
C		—	—	2/2	2/2	1/2	—	1/1	1/1	2/2	—	1/2	2/2	—	1/1

Allelic assignments were determined by Southern hybridizations to 11p gene probes and to probes from chromosomes other than 11p. For each probe, alleles are named with '1' as the larger restriction fragment and '2' or '3' as smaller fragments. '—' Indicates that normal and tumour samples were not analysed with a given probe, due to insufficient amounts of DNA. Chromosomes and the polymorphic probes used to mark these chromosomes are indicated at the top of the table. The clinical stage (T) and histopathological grade (t) of each tumour examined are indicated³². Southern hybridizations as described in the legend to Fig. 1 were performed with the following DNA probes: (1) antithrombin III (ATIII) (chromosome 1q, *Pst*I digest)³³; (2) carbamyl phosphate synthetase (CPSI) (chromosome 2p, *Bgl*II digest)^{13,33}; (3) DOSL C9 (chromosome 3, *Msp*I digest)³⁴; (4) collagen $\alpha 1$ (II) (chromosome 12q, *Hind*III digest)³⁵; (5) p7F12, p9D11, p9A7 (chromosome 13q, *Msp*I digests)³⁰; (6) pAW101 (chromosome 14q, *Eco*RI digest)¹⁴; (7) DOSL C3 (chromosome 15, *Msp*I digest)³⁴; (8) growth hormone (GH) (chromosome 17, *Msp*I digest)³⁶; (9) DOSL C6 (chromosome 18, *Taq*I digest)³⁴; and DOSL C2 (chromosome 20, *Msp*I digest)³⁴. Allele copy number was determined by densitometric analysis.

with duplications of the retained alleles, while a simple loss of 11p genes occurred in tumour cells of patients M, S2 and T, resulting in hemizyosity for 11p sequences.

Densitometric analysis also confirmed the marked reduction in intensity of one allele in each of the patients analysed in Figs 1 and 2. The hybridization signal corresponding to the deleted allele was diminished by 90%, 80%, 90%, 65% and 90% in patients A2, D, M, S2 and T, respectively, when tumour tissue was compared with normal tissue. Complete loss of the signal for the deleted allele was not observed. Two possible explanations for the remaining hybridization signal in the DNA from the tumour specimen were: (1) all tumour cells had lost an allele, but contaminating non-tumour cells were present within the tumour; or (2) the majority, but not all, of the tumour cells had lost an allele. In support of the first possibility, histological sections of tumour specimens from these patients revealed numbers of stromal cells roughly consistent with the signal remaining in the band corresponding to the deleted allele. However, possible cellular heterogeneity within the tumours created difficulty in ascertaining that the piece of tumour tissue used to prepare DNA had the same proportion of non-tumour and tumour cells as that examined for histopathological diagnosis. To address this potential problem, we extracted DNA from tissue sections in which the relative DNA contribution of tumour and non-tumour cells could be precisely determined. A piece of the tumour from patient D was frozen in OCT compound and 100 serial 6- μ m sections were cut with a cryostat. Sections 1, 51 and 99 were stained with haematoxylin and eosin for histological analysis and the remainder of the sections were used to prepare DNA using a technique described recently¹². The Southern blot obtained with the DNA from these sections is shown in Fig. 1, lanes D'. The result is quite similar to that obtained with the DNA isolated from a large piece of this tumour (Fig. 1, lanes D). Densitometry of the autoradiograph showed that the signal corresponding to the deleted allele was diminished by 83% when the DNA from the tumour sections was compared with

normal tissue from the same patient. Histological analysis revealed that 67% \pm 15% of the cells within these sections (3,800 cells in 26 fields examined) were tumour cells. Section 100 was stained with Feulgen, and microdensitometry was performed using a Leitz DAD video analysis system. This analysis revealed that the normal cells within the tumour had a relative DNA content of 1.0 \pm 0.15 while the tumour cells had a relative DNA content of 1.29 \pm 0.33. Hence, we expected that 86% (1.29 \times 67%) of the DNA extracted from these sections would be derived from tumour cells, and 14% derived from stromal cells within the tumour tissue. Comparison of the results of histological analysis and Southern blot analysis suggests that the residual hybridization of allele 1 in the tumour specimen from patient D was largely derived from contaminating stromal cells.

To obtain evidence that the changes we observed were specific to 11p and not simply widespread random alterations, we also examined DNA markers on chromosomes 1-3, 12-15, 17, 18 and 20. These data are summarized in Table 1. Interestingly, some changes were noted when genes from normal and tumour DNAs were compared on these chromosomes, but the incidence of these changes was less frequent than those seen on 11p. For example, we detected the loss of one carbamyl phosphate synthetase I allele (chromosome 2p)¹³ in each of the tumours from patients A2 and D (Table 1), and the loss of one pAW101 allele (chromosome 14q)¹⁴ in patient M. In addition, while reduction to homozygosity for chromosome 13 markers occurs in 60% of retinoblastomas¹, we did not observe the loss of any chromosome 13 markers in bladder tumours. Tumour tissues from patients M and T did exhibit a duplication of sequences on one chromosome 13 homologue, resulting in triploidy for chromosome 13 sequences (Table 1). Similarly, comparison of DNA obtained from tumour tissues of patients A1, D and P with DNA from their normal tissues showed hybridization intensities consistent with triploidy for chromosome 20 or chromosome 15 sequences in the tumour tissues. No other qualitative or quantitative chromosomal changes were detected in the other chromosomes

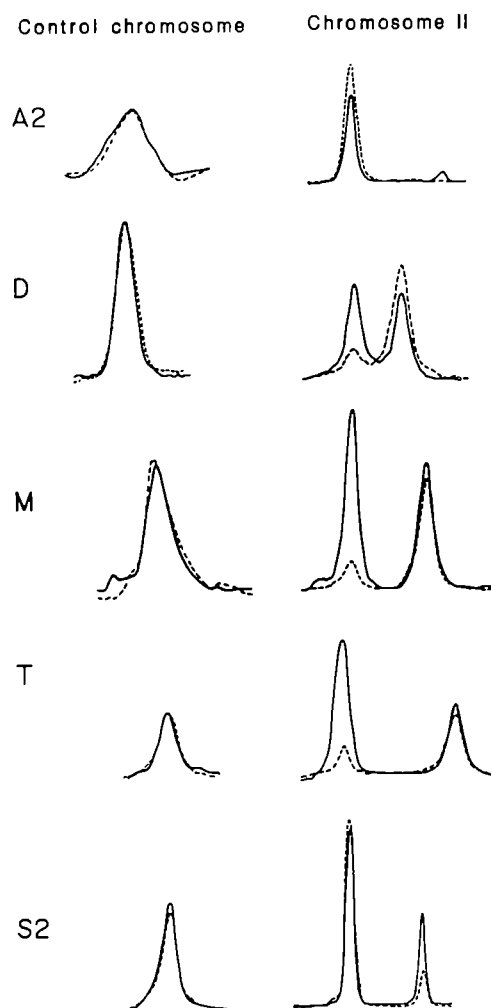


Fig. 2 Densitometric analyses of Southern hybridizations with 11p gene probes and of rehybridizations with non-chromosome 11 genes (control chromosome). Solid lines, normal lanes; broken lines, tumour lanes. Patient designations are indicated to the left. **Methods.** Southern blots (from Fig. 1) were freed of probe in alkali and rehybridized with control genes: (1) p9A7, a recombinant plasmid containing 0.95 kb of sequences derived from chromosome 13q³⁰ (patients A2, D and M; allele 2 was used for comparisons between the normal and tumour tissues in all three patients); or (2) pEP2, a recombinant plasmid containing coding sequences from the ATIII gene on chromosome 1q³¹ (patients S2 and T). Grain density was quantified using a Clifford Densicom model 445 densitometer. After normalizing the control scans for DNA loading, the 11p gene blots were re-scanned to produce the tracings shown.

examined (see Table 1). Hence, while loss of 11p sequences is certainly not the only genetic event that occurs in bladder cancer, our data would argue that such loss is relatively frequent in this cancer, and may be one of the important steps involved in development of this tumour.

We were unable to perform karyotypic analyses of the tumours studied. However, previous karyotypic analyses^{15,16} of transitional cell bladder carcinomas have revealed alterations of chromosome 1, 5, 8, 9, 11 and 13. Significantly, chromosomal losses involving 11p were seen in a subfraction of tumour cells from 3 of 9 patients in one study¹⁵ and at least 3 of 10 patients in another¹⁶. We observed a higher incidence of 11p losses than that revealed by karyotypic analysis. In addition, our results indicated that loss of 11p sequences occurred in the majority of the tumour cells from 5 of 12 patients. Thus the situation with bladder tumours is similar to that seen in Wilms' tumours, wherein analysis of DNA polymorphisms reveals a higher incidence of loss of 11p sequences than usually seen karyotypi-

cally¹⁷. These differences between the two analyses may be attributed to: (1) the difficulties inherent in cytogenetic studies of complex karyotypes from solid tumour specimens; (2) karyotypic analysis may be biased towards cells with the highest mitotic indices, while DNA polymorphism analysis represents an averaging of all cells within the tumour specimen; and (3) abnormal chromosome segregation which produces loss and reduplication events without gross loss of chromosomal material (such as that likely to have occurred in patients A2 and D) will not be detected karyotypically.

The chromosome 11 losses we observed were probably important for some stage of tumorigenesis in the five cases in which they occurred. This conclusion is strongly supported by our observation that the chromosomal loss, when observed, appeared to be present in nearly all cells of the tumour specimens (see Fig. 2). Hence, the losses did not simply occur in a small subclone of the tumour cell population; rather, the clone that lost 11p sequences apparently had a selective advantage sufficient to allow it to outgrow virtually all other cells.

There are at least two possible explanations for the growth advantage associated with loss of 11p sequences. First, it is possible that a normal cellular constraint of growth is present on chromosome 11p, and that the loss of this suppressor locus leads to activation of a normally repressed class of genes¹⁸. This mechanism does not seem to involve a simple dosage phenomenon. Bladder tumours from patients A2 and D and all of the Wilms' tumours in previous studies had a normal dosage of chromosome 11p arms, even though this diploid dosage was due to the loss of one chromosome 11p and the duplication of its homologue. Hence, for this explanation to be correct, one must postulate that two events had occurred in these cases: an inactivation of one chromosome 11p suppressor locus (by mutation, for example) followed by a loss of the chromosome 11p containing the normal suppressor locus, sometimes accompanied by the duplication of the abnormal 11p^{1,19}.

An alternative explanation for our results is that the events involving loss of 11p are simply correlated with another event that results in loss of growth control. In this model, the loss of 11p would itself have no intrinsic effect on the development of the tumour. For example, if a cell was prone to aneuploidy and chromosome 11 losses were not lethal to the cell, then some cells with a loss of chromosome 11 might also contain other alterations that could impart a growth advantage. This cell could then proliferate to produce a tumour with a clonal loss or loss and reduplication of 11p sequences. The fact that loss of 11p is only noted in 40–60% of bladder cancers and Wilms' tumours is consistent with this hypothesis.

In this context, however, examination of other tumours by molecular hybridization techniques does not reveal frequent loss of 11p. Of eight retinoblastomas studied for loss of 11p sequences, none showed loss of 11p^{1,20}. In addition, of 15 colon tumours examined, loss of 11p sequences was seen in only one tumour (E.R.F. and B.V., unpublished observations). Thus, loss of chromosome 11 sequences occurs frequently, and relatively specifically, in transitional cell carcinomas, Wilms' tumours, and other embryonal tumours. Its role in tumorigenesis will remain speculative, however, until the advent of studies which reveal the molecular effect of such loss.

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A polymorphic DNA marker linked to cystic fibrosis is located on chromosome 7

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Although cystic fibrosis (CF) is among the most common inherited diseases in Caucasian populations¹, the basic biochemical defect is not yet known. CF is inherited as an autosomal recessive trait apparently due to mutations in a single gene²⁻⁴, whence the efforts made to identify the genetic locus responsible by linkage studies. Two markers have recently been identified that are genetically linked to CF: one is a genetic variation in serum level of activity of the enzyme paraoxonase⁵, and the other is a restriction fragment length polymorphism (RFLP) identified with a randomly isolated DNA probe⁴. We report here that the genetic locus DOCRI-917 defined by the cloned DNA probe is located on chromosome 7.

The polymorphic locus DOCRI-917 is detected with the probe LAM4-917, a phage clone from the genomic library of Lawn *et al.*⁶ which is one of a set of probes identified by a random screening procedure for RFLPs⁷. It is a 17-kilobase (kb) single-copy human genomic DNA sequence polymorphic both at a *HindIII* site and a *HincII* site in Southern blot hybridizations. The probe is frequently informative in inheritance studies, having a polymorphism information content⁸ of 0.57.

The structure and inheritance of the locus are described by Tsui *et al.*⁴.

The marker locus DOCRI-917 was identified by DNA hybridization in rodent-human hybrid cell lines containing different human chromosome complements⁹. Hybridization of the probe to DNA from 17 hamster-human hybrids, 8 mouse-human hybrids and the parental human and rodent cell lines is shown in Fig. 1. As expected from the restriction map of the DOCRI-917 locus⁴, four fragments (8.9, 5.8, 5.0 and 3.6 kb) are detected in all lanes with complementary genomic sequences. (Restriction fragment length polymorphism at the DOCRI-917 locus is not observed with *EcoRI*.) The probe does not cross-hybridize with mouse or hamster sequences (lanes CH and M).

Comparison of the LAM4-917 hybridization results with the chromosome content of the 25 cell lines indicates that the DOCRI-917 locus is situated on chromosome 7. Probe hybridization conforms to the expected pattern of a chromosome 7 locus in every line, and shows multiple discordances with all other chromosomes (Table 1). In one cell line (CH13) that scored positive with the LAM4-917 probe, chromosome 7 was present at an earlier passage but was no longer detectable by karyotype analysis or by assay of the β -glucuronidase isozyme marker GUSB¹⁰. At least some chromosome 7 sequences were present, however, as shown by hybridization of CH13 DNA with a complementary DNA clone of the T-cell receptor β -chain gene¹¹, located on chromosome 7 (q3) (refs 12-15). As shown in Table 1, the hybridization results of LAM4-917 to all 25 lines were identical to those obtained with the T-cell receptor β -chain probe.

All chromosomes except chromosome 7 are excluded because they are present in one or more of the cell line DNAs not hybridizing to LAM4-917. It is particularly important to note that chromosome 2, even though the number of discordances is low, is excluded as the location of DOCRI-917 by the absence of hybridization of the probe in the line CH8. Chromosome 2 was detected in karyotype analysis of CH8 metaphase cells, the cells were positive for the chromosome 2 isoenzymes MDH1 and IDH1¹⁶, and hybridization to a DNA probe from chromosome 2 (D2S1¹⁷) is clearly detected in the same CH8 DNA sample. Exclusion of DOCRI-917 from the X and Y chromosomes was also confirmed by hybridization to a set of cell lines with sex chromosome aneuploidies (results not shown).

Because of its established genetic linkage to DOCRI-917, we conclude that the cystic fibrosis locus is also situated on chromosome 7. Determination of the location of the cystic fibrosis gene is an important step in identifying the primary genetic defect responsible for the disease. Any hypothesis that a particular genetic function is the primary lesion must satisfy the criterion of mapping to the same site as the cystic fibrosis trait. With the discovery of linkage between CF and the genetic markers PON⁵ and DOCRI-917⁴, the location of the disease gene is restricted to the 1% of the genome surrounding these markers. By chromosomal localization of DOCRI-917, that region is now mapped to chromosome 7. More specific localization of DOCRI-917 on chromosome 7 will be possible by testing other hybrid cell lines containing partial deletions of chromosome 7, *in situ* hybridization to metaphase chromosomes, and linkage mapping with other chromosome 7 markers. The observation that the DOCRI-917 locus is present in cell line CH13, which appears to retain only a part of chromosome 7 lacking GUSB (cen \rightarrow q22) (ref. 18) but retaining the T-cell receptor β -chain gene TCRB on 7q3 is consistent with a location on 7q, but other explanations of this result are possible. For example, more than one segment of chromosome 7 might have segregated with this line, or the DNA hybridization may simply be more sensitive than the other methods used to detect chromosome 7.

Population studies have supported the hypothesis that mutations in a single autosomal gene are responsible for CF^{2,3}. In addition, the observation of only 15% recombination between CF and the marker DOCRI-917 in 39 families indicates that the disease is attributable to mutations at the site we have identified on chromosome 7 in the vast majority of these families⁴. At

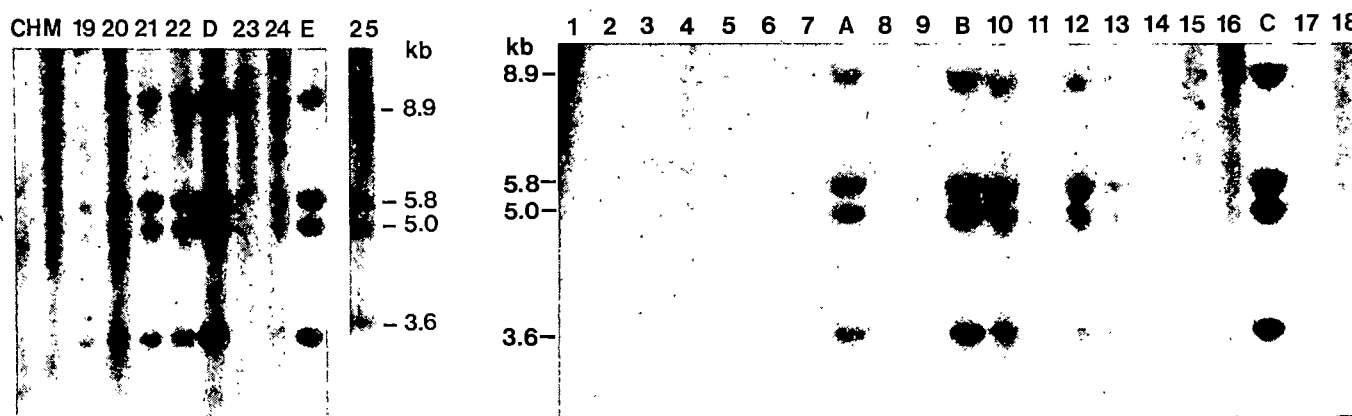


Fig. 1 Autoradiogram of hybridization of LAM4-917 to DNA of 25 somatic cell hybrids. Genomic DNA prepared from each hybrid cell line was digested with *Eco*RI, separated and transferred to DBM-filters¹⁹. DNA of the phage clone LAM4-917 was radioactively labelled with ³²P by nick-translation to a specific activity of 2×10^8 d.p.m. μg^{-1} . Hybridization was carried out for 20 h at 42 °C in 50% formamide, 0.6 M NaCl, 0.05 M Tris-HCl pH 7.6, 0.1% sodium pyrophosphate, 0.1% SDS, 0.04% Ficoll, 0.04% polyvinylpyrrolidone, 0.04% bovine serum albumin, 5% dextran sulphate, and 250 $\mu\text{g ml}^{-1}$ sonicated, denatured salmon sperm DNA. The filters were washed in $2 \times \text{SSC}$ at 20 °C for 15 min and twice at 65 °C for 15 min, in $0.2 \times \text{SSC}$, 0.2% SDS at 65 °C for 40 min, and finally in $0.1 \times \text{SSC}$, 0.1% SDS at 65 °C for 40 min. The filters were exposed to Kodak XAR-5 film with intensifying screens (Dupont Cronex Lightning Plus) at -70 °C. The lane numbers (1-25) correspond to the hybrid cell lines listed in Table 1. Lanes 1-17 are hamster-human hybrids, and lanes 18-25 are mouse-human hybrids. A-E are the human parental cell lines, CH is the chinese hamster cell line V79.4, and M is the mouse line C11DA.

Table 1 Chromosome localization of DOCRI-917

Cell line	Human chromosomes																						Translocation chromosomes	Hybridization				
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22		X	Y	TCRB	DOCRI-917	
1. CH1	-	-	-	+	-	+	-	+	/	+	-	+	+	/	-	-	-	/	-	/	+	-	-	-	Xp/2q	-	-	
2. CH2	-	-	+	+	-	-	-	+	-	-	-	-	-	+	-	-	-	/	-	/	+	-	-	-	+	Xp/2q	-	-
3. CH3	+	-	/	-	-	+	-	+	+	/	+	+	+	-	+	+	-	+	-	+	-	-	-	-	Xp/2q	-	-	
4. CH4	+	-	+	+	+	/	-	+	+	-	+	+	+	+	/	-	-	+	+	+	-	-	-	-	Xp/2q	-	-	
5. CH5	-	-	-	-	-	+	/	/	-	+	+	+	-	+	-	/	-	-	-	+	+	+	-	-	Xp/2q	-	-	
6. CH6	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	/	-	-	-	+	-	-	-	Xp/2q	-	-	
7. CH7	-	-	+	+	+	-	-	+	+	-	+	+	-	+	+	-	+	+	+	+	-	-	-	-	-	-	-	-
8. CH8	+	+	+	/	+	+	-	+	+	-	-	+	-	-	-	-	-	+	+	-	-	+	-	-	Xp/5q, 5p/Xq	-	-	
9. CH9	-	-	-	-	-	-	-	+	-	-	-	-	-	+	+	+	-	-	+	-	+	-	+	-	-	-	-	-
10. CH10	+	-	-	+	+	+	+	-	+	-	+	-	-	-	-	-	+	-	/	+	+	+	+	+	-	+	+	+
11. CH11	-	-	+	-	+	+	/	-	-	/	+	-	+	-	+	-	-	/	-	/	+	+	+	-	-	Xp/2q	-	-
12. CH12	-	-	-	+	+	+	+	/	-	-	+	+	+	+	+	+	-	+	-	+	+	/	+	-	-	Xp/2q	+	+
13. CH13	-	-	-	+	-	-	/*	/	-	-	+	+	-	-	-	-	-	+	+	-	-	-	+	-	-	Xp/2q	+	+
14. CH14	+	-	+	+	-	+	-	-	+	/	+	+	+	+	+	+	-	+	+	+	+	+	+	-	-	Xp/2q	-	-
15. CH15	-	-	-	+	+	+	-	/	-	+	+	-	+	+	-	/	-	-	+	-	+	-	/	-	-	Xp/2q	-	-
16. CH16	-	-	+	/	-	+	-	/	/	/	-	-	/	+	+	+	-	/	+	-	-	-	-	-	-	Xp/2q	-	-
17. CH17	-	-	-	-	-	+	-	-	-	-	+	+	-	-	-	-	-	-	-	+	/	/	+	-	-	-	-	-
18. M1	-	-	-	-	-	-	-	-	-	+	-	-	-	-	+	-	-	-	/	+	+	-	+	/	-	-	-	-
19. M2	+	+	-	+	+	-	+	+	-	/	+	+	-	-	+	+	+	+	+	+	+	+	+	/	-	Xp/5q	+	+
20. M3	-	+	+	/	-	-	+	+	-	-	-	+	+	/	-	+	+	+	+	+	+	+	+	-	-	5p/Xq	+	+
21. M4	/	+	+	-	+	+	+	+	-	+	+	+	+	-	-	+	+	+	+	+	+	+	+	-	-	-	+	+
22. M5	-	+	+	/	+	+	+	-	-	-	+	-	-	-	-	-	+	/	+	+	+	+	/	-	-	-	+	+
23. M6	-	-	+	/	-	+	-	+	-	-	-	+	+	+	-	/	+	-	+	+	+	-	/	-	-	-	-	-
24. M7	/	/	+	+	+	/	+	+	-	-	+	-	+	/	-	-	+	-	+	/	-	-	/	-	-	-	+	+
25. M8	-	+	+	-	-	+	+	-	-	-	+	+	-	-	-	-	+	-	-	-	-	/	-	+	-	Xp/5q, 5p/Xq	+	+
Discordant																												
DOCRI-917 (%)	39	17	50	40	28	57	0	60	57	55	36	48	50	68	54	43	21	29	48	45	59	29	32		0			

Chromosomal content of hybrid cell lines was determined by cytogenetic and isoenzyme analysis. The panel of hybrid cell lines was generated and characterized by Nguyen Van Cong, Dominique Weil and Catherine Finaz, Unité de Recherches de Génétique Médicale, INSERM U12, Paris, France, and is described elsewhere⁹. Symbols: +, chromosome detected in more than 30% of cells; -, chromosome not detected; /, chromosome detected in less than 30% of cells, not scored for mapping. Three of the human parental cell lines contained reciprocal chromosomal translocations^{20,21} retained by some hybrids: Xq/2q (Xqter → p22:2q32 → 2qter), Xp/5q (Xpter → q21:5q11 → qter), and 5p/Xq (5pter → q11:Xq21 → qter). Hybridization results for DOCRI-917 are from the autoradiogram in Fig. 1. The TCRB gene is detected by hybridization with the T-cell receptor β -chain probe¹¹ with the same cellular DNA samples. * Possible chromosome rearrangement, see text.

present, we cannot exclude the possibility that mutations in genes not linked to DOCRI-917 and PON are responsible for CF in a small percentage of families.

The localization of DOCRI-917 to chromosome 7 greatly facilitates the identification of other RFLP markers linked to CF. Polymorphic probes already assigned to chromosome 7 can be tested for linkage to CF, and collection of additional RFLP markers for this purpose can be accelerated by drawing probes from chromosome 7-specific libraries. Such RFLP markers will

contribute to increasing the resolution of the linkage map of the CF region, and ultimately to the identification of the CF gene itself.

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Note added in proof: Our recent linkage analysis shows that both CF and DOCRI-917 are closely linked to the pro α 2(1) collagen gene (7q21-q22) (ref. 22 N. Buchwald et al., in preparation).

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A closely linked genetic marker for cystic fibrosis

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Cystic fibrosis is a recessive genetic disorder, characterized clinically by chronic obstructive lung disease, pancreatic insufficiency and elevated sweat electrolytes; affected individuals rarely live past their early twenties. Cystic fibrosis is also one of the most common genetic diseases in the northern European population. The frequency of carriers of mutant alleles in some populations is estimated to be as high as 1 in 20, carrying a concomitant burden of about one affected child in 1,500 births. Because little is known of the essential biochemical defect caused by the mutant gene, a genetic linkage approach based on arbitrary genetic markers and family studies is indicated to determine the chromosomal location of the cystic fibrosis (CF) gene. We have now obtained evidence for tight linkage between the CF locus and a DNA sequence polymorphism at the *met* oncogene locus. This evidence, combined with the physical localization data for the *met* locus presented in the accompanying paper¹, places the CF locus in the middle third of the long arm of chromosome 7, probably between bands q21 and q31.

Appropriate families are required for genetic linkage analyses with recessive disease loci. For this study, we sampled and tested 13 families with multiple affected offspring. Siblings were characterized at cystic fibrosis clinical centres as affected or unaffected based on quantitative sweat chloride determination as well as other clinical features. Several of the families were from Utah, but most came from different regions in the United States.

We estimate that more than 100 loci have been tested for linkage in CF, including 21 probes from our own laboratory, with negative results. However, Eiberg et al. have reported evidence of linkage between the CF locus and the gene for the enzyme paroxonase², and recently Tsui and others have obtained evidence for genetic linkage with a DNA marker (L.-C. Tsui, personal communication). Both of these linkages are rather

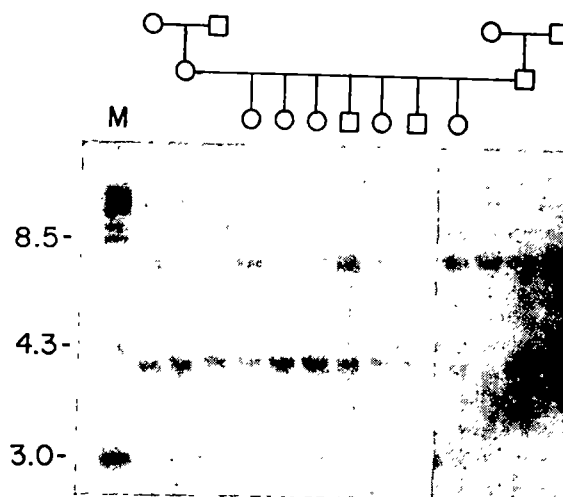


Fig. 1 Segregation of the *TaqI* polymorphism in a complete three-generation family. DNAs from the indicated individuals were digested with the restriction enzyme *TaqI*, electrophoresed in agarose and transferred by the method of Southern to a nylon filter (Micon Separations, Inc.). The filters were hybridized⁹ with the clone *metH* (refs 1, 3). The polymorphisms were found by examination of a panel of DNAs from six unrelated individuals digested with the restriction enzymes *MspI*, *TaqI*, *EcoRI*, *HindIII*, *PstI* or *BclI*. Only *MspI* and *TaqI* revealed polymorphism. Individuals digested with *TaqI* yielded fragment lengths of either 7.5 kb (allele 1) alone or 4.0 kb (allele 2) alone, or both 7.5 and 4.0 kb. Digestion with *MspI* revealed individuals with fragment lengths of 2.3 and 1.8 kb, or only one of the two. In six informative families with large sibships, we found no exceptions to mendelian inheritance. A survey of 60 unrelated individuals gave a frequency of 0.56 for the 7.5-kb *TaqI* allele and the 2.3-kb *MspI* fragment, and 0.44 for each of the two smaller fragments. In all cases where phase could be distinguished, the large fragments were found together as a single haplotype, indicating a high degree of linkage disequilibrium in this marker system.

loose (10% and 15% recombination, respectively) but they do begin to pave the way for a more precise localization. Klinger has also reported suggestive, but not definitive, evidence for linkage of cystic fibrosis to a genetic marker located on the short arm of chromosome 21 in two large Amish pedigrees. However, the linkage was not evident when a collection of outbred nuclear families was examined (Klinger, personal communication). To develop a genetic marker at the *met* locus, we cloned fragment H (refs 1, 3), a 1.6-kilobase (kb) *SalI/EcoRI* fragment, into pBR322. Figure 1 shows the inheritance of a polymorphic *TaqI* restriction fragment revealed by this probe in a complete three-generation family. An *MspI* fragment also showed polymorphism with this probe. Of 60 unaffected unrelated individuals examined, 36 were heterozygous. However, we have seen only two haplotypes thus far.

At least one parent was heterozygous at the *met* locus in 12 of the 13 CF families investigated. Linkage tests between the CF locus and the *met* locus were performed with the computer program LINKAGE (ref. 4). Our results are reported in Fig. 2 and Table 1. As shown in Fig. 2, the LOD score reaches a maximum of 8.65 at a recombination value of 0, indicating no evidence for recombination. A LOD score of 8.65 corresponds to odds of $4 \times 10^8:1$, favouring the hypothesis of complete linkage over that of independent segregation. We also verified that in each family the LOD score is maximal under the hypothesis of complete linkage. As in all estimation situations, our sample estimate of the recombination rate between these two loci may depart from the true, unknown value because of sampling error. As indicated in Fig. 2, however, the true recombination frequency is unlikely to be $>5\%$. Our data therefore indicate tight linkage between cystic fibrosis and the *met* gene in these families.

Table 1 shows the composition, the genotypes at the *met* locus and the LOD score for each family. Under the hypothesis of

Table 1 Summary of family data

Family	Parents	Offspring	LOD score
1409	12×11	3A (11)	0.60
1414	12×22	3A (12)	0.60
1415	12×12	3A (11) 1N (12)	1.33
1422	12×11	3A (12)	0.60
1425	12×11	5A (12) 2N (12, 11)	1.15
1426	22×12	3A (12)	0.60
1427	12×12	3A (11) 4N (12)	1.70
1438	11×12	2A (11)	0.30
1442	12×11	3A (11)	0.60
1446	11×12	3A (11)	0.60
774	11×12	2A (11)	0.30
1378	22×12	3A (22) 2N (22)	0.25
1436	11×22	3A (12)	0.00

Genotypes at the marker locus reported for each family member. The LOD score for each informative family at a recombination value of 0 is also reported, allowing a closer inspection of the contribution of each family to the linkage evidence. Assuming that all parents are heterozygous at the CF locus, all but two (families 1415, 1427) are single backcrosses with respect to the test locus. In such families, each affected offspring contributes terms equal to θ and $1 - \theta$ under each parental phase respectively. Under complete linkage, phase information can be derived from a single affected individual. It follows that each additional individual will contribute 2 units to the odds ratio, or equivalently 0.301 to the LOD score, while the penalty of having to infer phase amounts to 0.301 LOD unit in each family. In two instances of such crosses, unaffected siblings were available for testing. In family 1425, five affected siblings have genotype 12, whereas two unaffected siblings have genotypes 12 and 11, respectively. Without the unaffected siblings, the LOD score would have been 1.20 instead of 1.15. The two unaffected siblings, while compatible with the hypothesis of no recombination, have discordant genotypes at the test locus and contribute terms $(2 - \theta)(1 + \theta)$ under each parental phase, accounting for -0.05 in LOD units. In family 1378, the contribution of three affected offspring is offset by two unaffected siblings with the same genotype at the *met* locus, each unaffected sibling accounting for terms $(2 - \theta)$ and $(1 + \theta)$ under each phase. Family 1427 is a double intercross and contributes more support to the hypothesis of complete linkage than any other family. This results from the increased contribution of affected individuals of genotype 11 in this intercross as opposed to the backcrosses, as well as from the information provided by the four unaffected siblings of genotype 12. Once an affected individual has yielded phase, each additional affected sibling of genotype 11 contributes $(1 - \theta)^2$, or 0.602 LOD units, while each unaffected sibling of genotype 12 contributes $2(1 - \theta + \theta^2)$, or 0.125, to the overall LOD score. Family 1415 is similar, but includes only one unaffected sibling of genotype 12.

complete linkage, we can derive the parental phases from the genotypes of the affected offspring. This reveals that 19 of the 26 parental chromosomes carrying the CF allele also carry allele 1 at the *met* locus, while only 11 of 26 parental chromosomes carrying the normal allele at the CF locus carry allele 1 at the *met* locus, which is marginally significant ($\chi^2_1 = 3.86$, $p = 0.05$). The allelic distribution at the *met* locus among the latter chromosomes does not differ significantly from that observed among 60 chromosomes tested in a random Utah control panel ($\chi^2_1 = 0.98$). When these chromosomes carrying a normal allele at the CF locus are pooled and contrasted to the chromosomes carrying the CF deleterious allele, heterogeneity with respect to the *met* alleles 1 and 2 is no longer significant ($\chi^2_1 = 2.71$). Judgment regarding possible allelic association must await the characterization of additional CF families.

The apparent close genetic linkage found in family studies suggests an upper limit of 5% on the genetic distance between the CF locus and the *met* locus. The absence of recombinants between the CF locus and the *met* locus in our study does not support the hypothesis that cystic fibrosis can be caused by mutations at more than one locus. However, ours is still a limited sample and it is important to test many more families to place a more restrictive upper limit on the possible frequency of cystic fibrosis caused by mutations at other loci.

Although the *met* locus is potentially useful as a prenatal diagnostic marker for cystic fibrosis in families known to be at risk, several additional data must be developed before such application. First, more CF families with large sibships must be tested, to refine the genetic distance as well as to detect locus heterogeneity. Observation of a subset of families showing no linkage to the *met* locus could reveal locus heterogeneity. These data will be essential for providing quantitative estimates of

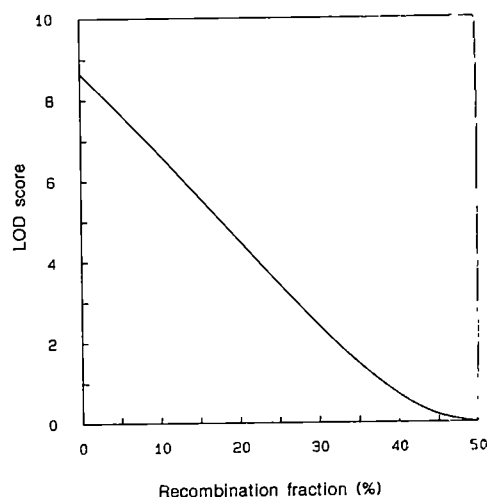


Fig. 2 LOD score for linkage between cystic fibrosis and the *met* locus as a function of recombination fraction. A recessive model with complete penetrance was assumed for cystic fibrosis, with a frequency of 0.025 for the deleterious allele. The results were unaltered over a wide range of allelic frequencies. The test marker was treated as a co-dominant, two-allele system, with gene frequencies of 0.56 and 0.44 for alleles 1 and 2, respectively. The *TaqI* polymorphism was scored as the primary genetic marker and was corroborated by the *MspI* polymorphism. The LOD score is computed as the decimal logarithm of the probability of the observations for a given value of the recombination fraction divided by the corresponding probability under the assumption of no linkage. This probability ratio measures our degree of belief in the hypothesis of linkage at the assumed value relative to the hypothesis of independent segregation. Rather than a standard error based on large sample theory, the use of an empirical confidence region based on the observed LOD score distribution has been recommended¹⁰. A one-unit support limit is obtained by finding that value of the recombination fraction for which the LOD score is decreased by one unit, defining a range of the recombination value for which the odds remain within 10 to 1 of the maximum. This yields an empirical bound for the recombination fraction of 0.051 in our sample. Values for recombinant fraction θ of 0, 0.01, 0.05, 0.10, 0.20, 0.30 and 0.40 produced respectively values for $z(\theta)$ of 8.65, 8.46, 7.66, 6.64, 4.52, 2.41 and 0.70.

individual risk. Second, the extent of polymorphism must be improved so that the marker locus has more than two alleles. At present, many individuals at risk would be unable to obtain useful information. Third, if, as we expect, some recombinants between the *met* and CF loci are found, it will be important to develop a genetic marker locus on the other side of the CF locus to detect recombination events in the region. Until these conditions are met, diagnostic applications are premature.

The fact that no recombinants between the *met* gene locus and the CF locus have yet been identified suggests that the two are indeed very close and even raises speculation that the *met* gene might actually be the cystic fibrosis gene. Furthermore, because the product of the *met* gene is a membrane-spanning receptor protein showing tyrosine kinase homology¹, characteristics which might also be expected of a gene causing a chloride ion-exchange defect, in retrospect *met* might have been considered a candidate gene for the disease. However, although we cannot exclude the *met* gene as a candidate for the CF gene, aetiological involvement of the *met* gene in cystic fibrosis is unlikely: the close linkage still permits a span of more than 1,000 kb within which to place the CF gene and as many as a hundred genes may be located within so wide a region. It is nonetheless interesting that the *met* gene bears homology with the insulin receptor¹, located on chromosome 19 very near the low-density lipoprotein (LDL) receptor⁵; this homology may suggest that these are members of a clustered family of genes. By this reasoning, although not itself the CF gene, *met* could

open the way to identifying a family of closely linked genes of which one may be the gene for cystic fibrosis.

The most significant aspect of the localization of the CF gene is the impetus it provides to efforts to identify and clone the gene. New techniques make it feasible to cover the large distances that are likely to be involved. Pulse-field gel electrophoresis⁶ and new, rapid 'walking' techniques⁷ suggest approaches to moving closer to the gene. Recent advances in cell culture technology that permit *in vitro* growth of cells which maintain the CF phenotype⁸ suggest the possible invention of protocols that could provide functional assays for regions that might encompass the gene. We hope that identification of the CF gene will permit identification and analysis of the biochemical pathway which is perturbed by mutations in the gene, and will suggest rational means of intervention and management of this disease.

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Localization of cystic fibrosis locus to human chromosome 7cen-q22

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Cystic fibrosis (CF) is the most common genetic disease in Caucasian populations, with an incidence of 1 in 2,000 live births in the United Kingdom, and a carrier frequency of approximately 1 in 20. The biochemical basis of the disease is not known¹, although membrane transport phenomena associated with CF have been described recently². Consanguinity studies have shown that the inheritance of CF is consistent with it being a recessive defect caused by a mutation at a single autosomal locus³. Eiberg *et al.*⁴ have reported a genetic linkage between the CF locus and a polymorphic locus controlling activity of the serum aryl esterase paraoxonase (PON). The chromosomal location of PON, however, is not known⁴. Linkage to a DNA probe, DOCR1-917, was also recently found at a genetic distance of ~15 centimorgans (L.-C. Tsui and H. Donniss-Keller, personal communication), but no chromosomal localization was given. Here we report tight linkage between the CF locus and an anonymous DNA probe, pJ3.11, which has been assigned to chromosome 7cen-q22.

The use of DNA probes allows the determination of linkage between a restriction fragment length polymorphism (RFLP) and a mutated locus causing pathology, even when the biochemical defect is unknown. We have used this approach successfully to analyse sex-linked diseases such as Duchenne mus-

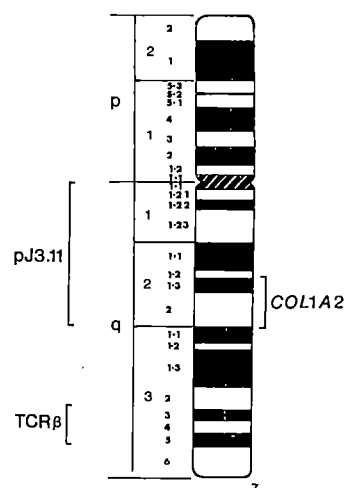


Fig. 1 Map of human chromosome 7, showing the chromosomal locations of probes *TCRβ* (ref. 9), *COL1A2* (ref. 25) and pJ3.11 (ref. 18).

cular dystrophy⁵; it has also been used to study previously unassigned autosomal dominant diseases such as Huntington's chorea⁶ and adult onset polycystic kidney disease⁷. However, although linkage has been attempted with DNA markers to autosomal recessive diseases of unknown biochemical aetiology, only exclusions have been achieved to date.

There have been few clues to the chromosomal localization of the CF locus. Mayo *et al.*⁸ suggested that the CF locus might be localized on chromosome 4, based on data from cell hybrids expressing a ciliary dyskinesia factor, but this was subsequently shown to be unlikely by exclusion mapping with several DNA and protein markers⁹. Two different families have been reported to show both chromosomal anomalies and CF; the study of one of these families suggested that the CF locus mapped to 5p, whereas in the other family it was mapped to 13q34 (ref. 10). We investigated the latter family by mapping the gene for clotting factor X to 13q34 (ref. 11) and then excluded CF from linkage to factor X¹². We have also excluded the candidate gene coding for complement component 3 by showing that alleles for an

Table 1 LOD scores at various recombination fractions (θ) for the relationships between the CF locus (CF) and the locus defined by pJ3.11 and between CF and *TCRβ*

Marker	No. of meioses	$\theta = 0$	0.05	0.10	0.15	0.20	0.25	0.30	0.35	0.40	0.45
CF versus J3.11	27	5.24	4.52	3.80	3.09	2.41	1.77	1.20	0.77	0.33	0.11
<i>TCRβ</i> versus CF	22	—∞	1.87	2.06	1.87	1.54	1.17	0.80	0.47	0.22	0.06

Combined LOD scores were calculated using the computer program package LINKAGE as described by Lathrop²⁴.



Fig. 2 Segregation of the *Msp*I polymorphism defined by pJ3.11 with the CF locus in a family with three affected (solid symbols) and three unaffected (open symbols) children. The RFLP alleles are 4.2 kilobases (kb) (A1) and 1.8 kb (A2) long; the frequency of the rare allele A1 is 0.2 (ref. 26).

Methods. DNA (5 µg) from 10 informative families with two or three affected children was digested with *Msp*I and fractionated by electrophoresis on 0.8% agarose gels by standard methods⁹. Fractionated DNA was transferred to Hybond membranes (Amersham International) and hybridized⁹. The probe used was the 500-bp *Hind*III-*Eco*RI fragment from the insert of pJ3.11, labelled to a specific activity of 1×10^9 d.p.m. µg⁻¹ by synthesis using random oligonucleotide primers. Blots were washed to a final salt concentration of 0.15 M NaCl in the presence of 0.2% SDS. Autoradiography was for 16 h at -70° using double-intensification screens.

RFLP detected by this sequence do not co-segregate with cystic fibrosis in families with multiple affected sibs¹³. Several groups have reported extensive linkage exclusions between the CF locus and protein and DNA markers¹⁴⁻¹⁶, which in total exclude ~40% of the human genome.

We have tested for linkage between the CF locus and multiple markers on a single human chromosome by multipoint linkage analysis¹⁷. As part of our attempt to study human chromosomes for which few exclusions had been obtained for cystic fibrosis, we have examined the linkage relationships of the two DNA markers located on chromosome 7 to the CF locus. These are the anonymous DNA segment pJ3.11, which defines an *Msp*I polymorphism and has been mapped to 7cen-q22 by somatic cell hybrid panels¹⁸, and the T-cell receptor β -chain (*TCR* β) gene which defines *Bgl*II polymorphism and maps to 7q3 (ref. 19). These localizations are summarized in Fig. 1.

The linkage relationships between the two markers and the CF locus are shown in Table 1. The maximal LOD score between the locus defined by pJ3.11 and the CF locus calculated for combined sexes is 5.24 at a recombination fraction (θ) = 0 (99% confidence interval 0-13 centimorgans). A LOD score of 3.0 is accepted as a sufficient statistic to prove synteny between two loci²⁰. No recombinants between the probe and the CF locus have been observed in 27 meioses. A typical pedigree is shown in Fig. 2. All informative pedigrees and typings are available from the authors.

TCR β (7q3) shows significant linkage (for two syntenic loci²¹) to the CF locus at a genetic distance of 10 centimorgans. We have also demonstrated that the collagen locus *COL1A2* is linked to the CF locus at a genetic distance of ~10 centimorgans²², suggesting that *COL1A2* (7q22) and *TCR* β (7q3) may flank the CF locus, as they are not themselves closely linked. We are presently analysing the linkage between pJ3.11, *TCR* β and *COL1A2* in large reference pedigrees (Centre d'Etude Polymorphisme Humain), as well as in cystic fibrosis families which have been typed for *PON*.

Genetic heterogeneity of the locus mutated in cystic fibrosis (as opposed to multiple mutations causing a defect at a single locus) would be demonstrated by the observation of heterogeneity in the linkage relationship in different families between a given close marker and the CF locus. The informative families in this study originated from Australia, Eire, France,

West Germany, United Kingdom and Pakistan. All the families show tight linkage between the pJ3.11 probe and the CF locus. It would be of interest to study these probes in the Amish kindred reported by Klinger²³, as there are data indicating differences in linkage for that family compared with those studied here (K. Klinger, personal communication). A more extensive analysis covering different populations is in progress.

The pJ3.11 probe is presently informative in approximately 40% of matings and, given the tight linkage demonstrated here, would provide accurate heterozygote detection and antenatal diagnosis of cystic fibrosis in some cases. However, such diagnoses will be facilitated markedly when the availability of a set of informative and ordered flanking markers (*PON*, pJ3.11, *COL1A2*, and *TCR* β) has been confirmed.

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The human *met* oncogene is related to the tyrosine kinase oncogenes

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The *met* oncogene was previously isolated from a chemically transformed human cell line, MNNG-HOS (refs 1, 2). Recent evidence has demonstrated that two classes of transcripts are expressed from the *met* proto-oncogene locus. The *met* oncogene, however, expresses an aberrant RNA which has sequences in common with both transcripts. We now report partial nucleotide sequencing of the human *met* oncogene and show that *met* is

related to the protein kinase oncogenes and growth factor receptors. The *met* nucleotide sequence is not identical to that of any published gene, and it is more closely homologous to the tyrosine kinases than to the serine/threonine kinases. Within the tyrosine kinase family, the sequenced *met* domains are most closely related to the human insulin receptor and the viral *abl* gene. *In situ* chromosome hybridization has mapped *met* to human chromosome 7 band 7q21-q31, a location distinct from that of other kinases. This is also a region associated with nonrandom chromosomal deletions observed in a portion of patients with acute non-lymphocytic leukaemia. The accompanying paper³ shows that this chromosomal locus is also tightly linked with the human hereditary disease cystic fibrosis.

To characterize the *met* oncogene and its protein product(s), we have sought to determine the nucleotide sequence of coding portions of the locus. The sequences required for transformation by *met* are contained within 35 kilobases (kb) of human genomic DNA and NIH 3T3 cells transformed with *met* express a 6.5-kb messenger RNA^{1,4}. Initial attempts to isolate a complementary DNA copy of this RNA generated a 2.0-kb clone. However, the partial nucleotide sequence indicated that this clone contains only untranslated sequences (M.P., data not shown). As an alternative approach, a 9-kb fragment was cloned into a retrovirus vector derived from Moloney sarcoma virus (Fig. 1).

Retroviral vectors have been shown to process segments of genomic DNA, generating cDNA-like copies⁵. We hoped that by cloning a segment of *met* genomic DNA into such a vector we could recover a clone devoid of intervening sequences. An *S*₁ nuclease experiment revealed that passage of this segment of *met* genomic DNA through a retroviral intermediate resulted in the excision of at least one intervening sequence; the recovered clone protected a fragment 1–200 bases longer than the corresponding segment of genomic DNA (data not shown). Nucleotide sequencing of the recovered clone and the corresponding portions of genomic DNA revealed an open reading frame 126 amino acids long (Fig. 2A). When this putative *met* amino-acid sequence was used to search an amino-acid sequence database, homology with several tyrosine kinase oncogenes and growth factor receptors was revealed (Fig. 2B).

In addition, we have also determined the nucleotide sequence of a 1.1-kb fragment of genomic DNA from the middle of the *met* oncogene (fragment D) which is known to hybridize with *met* mRNA transcripts². This sequence contained an open reading frame bounded by splice acceptor and donor signals which shows dramatic homology to the tyrosine kinases (Fig. 2). Figure 2B compares the amino-acid sequence of the two open reading frames with several members of the tyrosine kinase family. The putative exon is identical in 18/23 residues with the human insulin receptor^{6,7} and in 15/23 amino acids with the virus *abl*

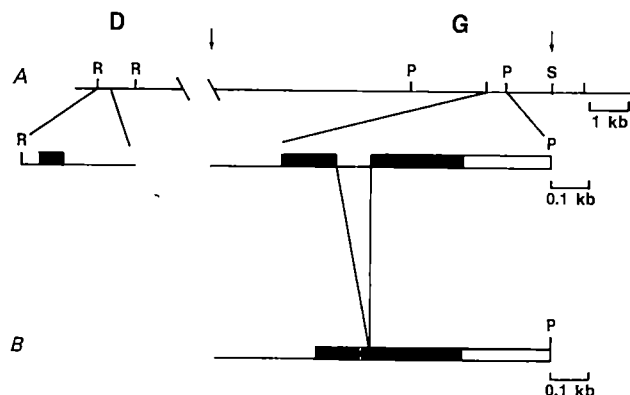


Fig. 1 Sequencing strategy of the *met* oncogene. **A**, Genomic DNA located at the 3' end. Filled areas depict coding regions or putative exons. **D** and **G** refer to fragments described previously^{1,2}. Both strands of the expanded regions were sequenced by the chain-termination method²⁶. The 9-kb *SalI* fragment inserted into the retrovirus vector is depicted by the arrows. **B**, *met* sequences contained within the spliced clone recovered. **R**, *EcoRI*; **P**, *PstI*; **S**, *SalI*.

Methods. The 9-kb *SalI* fragment was cloned into pGV16 (ref. 27), which was modified to accept *SalI* fragments by inserting a *SalI* linker. Virus was rescued from this plasmid by co-transfection with an equal amount of pMOV-3 plasmid²⁸ into NIH 3T3 cells. Infected NIH 3T3 cells were subsequently selected for resistance to G418 using 500 $\mu\text{g ml}^{-1}$ Geneticin (Gibco). The viral DNA from the transfected cells was recombined by fusing G418-resistant NIH 3T3 cells with COS cells using polyethylene glycol as described previously²⁹. Hirt supernatants³⁰ were prepared after 72 h and used to transform *Escherichia coli* strain HB101 (BRL) using the procedure described by Hanahan³¹ and selected on kanamycin (35 $\mu\text{g ml}^{-1}$). Only colonies which hybridized to the *G* fragment probe (see text) were analysed.

gene⁸. This region is highly conserved among all members of the tyrosine kinase family⁹, and moderately conserved between other kinases such as *mos* (ref. 10) (Fig. 2B), *raf* (ref. 11), the yeast CDC28 protein¹² and cyclic AMP-dependent kinase¹³. Although the two exons at the C-terminus of *met* contain a region which is less well conserved among kinases⁹, certain portions of this domain are conserved among all members of the family, including *met* (Fig. 2B).

We have previously demonstrated that although *met* maps to chromosome 7, it is not the *erbB*/epidermal growth factor (EGF) receptor gene, which is located at chromosome 7 p12-p14 (refs 1, 14). To map the location of *met* on chromosome 7 more precisely, we performed *in situ* chromosomal hybridization. A ³H-radiolabelled *met* probe was hybridized to metaphase cells

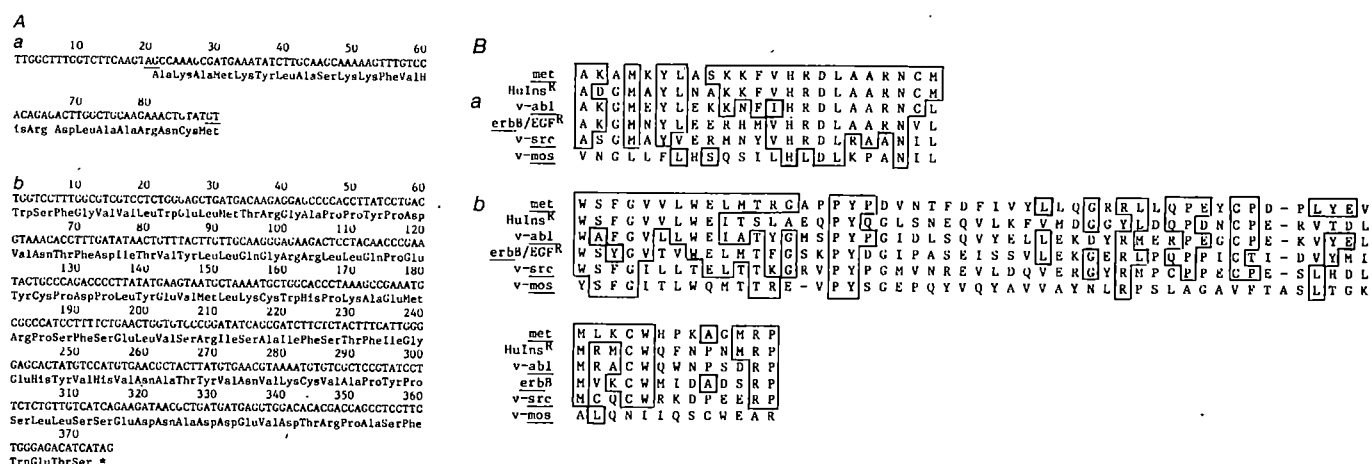


Fig. 2 Partial nucleotide sequence of the *met* oncogene. **A**, The nucleotide sequence and inferred amino-acid sequence of portions of *met* are shown: **a**, putative exon from **D** fragment; **b**, open reading frame of the last two exons. Putative splice signals are underlined. **B**, Comparison of the *met* amino-acid sequence with corresponding regions of the human insulin receptor (Hu InsR)^{6,7}, viral *abl* oncogene⁸, human EGF receptor³², and viral *src* and human *mos* proto-oncogenes^{10,33}.

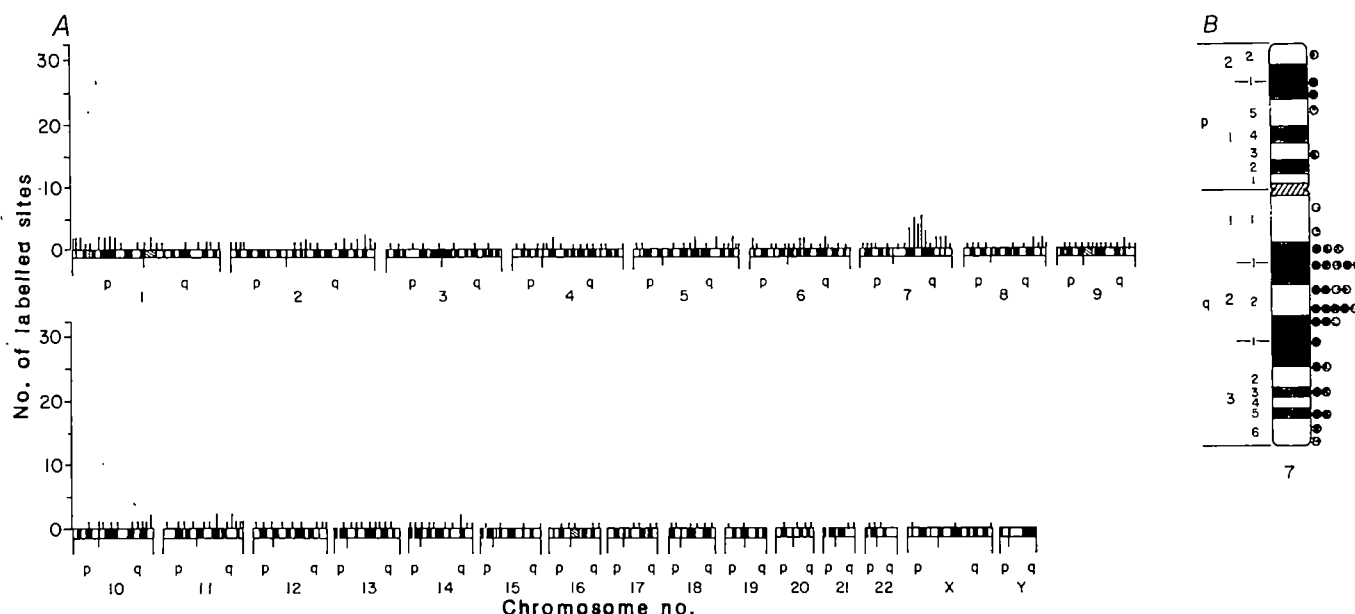


Fig. 3 *In situ* chromosomal hybridization of the *met* oncogene. **A**, Distribution of labelled sites in 100 normal metaphase cells. Human metaphase cells prepared from phytohaemagglutinin-stimulated peripheral blood lymphocytes were hybridized with the ^3H -labelled E probe¹. Specific labelling was observed on chromosome 7 at bands q21–q31. **B**, Distribution of labelled sites on chromosome 7. Of 100 metaphase cells examined, 19 (19%) were labelled on bands q21–q31 of chromosome 7. Of a total of 36 grains on chromosome 7, 21 or 58% were located on q21–q31; these sites represented 8.2% (21/256) of all labelled sites ($P > 0.005$). A modification of the method of Harper and Saunders was used^{34,35}.

from phytohaemagglutinin-stimulated peripheral blood lymphocytes. Figure 3A shows the distribution of grains observed in 100 metaphase cells. A significant clustering of grains was observed on the long arm of chromosome 7 at bands q21–q31 ($P < 0.005$); no other chromosome showed specific labelling. Of the 100 metaphase cells examined, 19 were labelled on bands q21–q31 of one or both of the chromosome 7 homologues (Fig. 3B); these sites represented 8.2% (21/256) of all labelled sites. The largest cluster of grains was observed at band 7q22. Specific labelling was also observed at this chromosomal region when *met* was hybridized to metaphase cells from peripheral blood lymphocytes obtained from a second individual. Of 50 metaphase cells examined, 10 showed label on one or both chromosome 7 homologues, representing 7.1% (12/169) of all labelled sites. These results confirm the location of this gene on the long arm of human chromosome 7 and refine the location to band q21–q31.

We conclude that the *met* oncogene is a member of the tyrosine kinase family, although analysis of the *met* protein product will be required to establish whether it possesses this activity. Analysis of *met*-related mRNAs has revealed that the transforming gene expresses a hybrid truncated mRNA⁴. Thus, we speculate that the *met* oncogene transforms cells by expressing a truncated form of the kinase encoded by the proto-oncogene. A similar mechanism appears to be responsible for the activation of both the *v-erb-B* and *c-erb-B*/EGF receptor genes^{15,16}, and the *bcr/abl* gene in chronic myelogenous leukaemia¹⁷.

The homology between *met* and the kinase domain of the insulin receptor is intriguing. Insulin is a member of a gene family which includes insulin-like growth factors I and II and nerve growth factor¹⁸. Similarly, there are several distinct receptors for insulin-related mitogens¹⁹. It has not escaped our attention that the *met* class I gene product may encode a receptor for an insulin-like peptide. However, *met* is also closely related to *abl*. Identification and characterization of the *met* protein(s) should help address these interesting questions.

Nonrandom deletions of the long arm of chromosome 7 are frequently observed in the leukaemic cells of patients with acute non-lymphocytic leukaemia (ANLL). Interestingly, *met* was isolated from a human cell line (HOS) transformed with the chemical carcinogen *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine, and increasing evidence now relates the development of ANLL

to mutagen exposure both in patients subjected to cytotoxic therapy for a primary malignancy and in patients exposed to environmental carcinogens^{20,21}.

It is notable that recurring abnormalities involving chromosome 7 are common in such patients. These abnormalities include loss of a whole chromosome 7 or of part of the long arm of this chromosome, del(7q) (refs 20, 22). It may be significant that *met* is located in the region of the proximal breakpoint of the interstitial deletions of 7q observed in patients with ANLL²³.

In the accompanying paper, White *et al.*³ describe restriction fragment length polymorphisms (RFLPs) from the *met* locus which were developed for examining ANLL patients. They find that these RFLPs are tightly linked to the hereditary disease cystic fibrosis (CF). The *in situ* chromosomal mapping of *met* thus localizes the CF locus in man. At the very least then, *met* is a marker for CF, a disorder that is speculated to involve an ion-channel imbalance²⁴. However, we show here by sequence homology that *met* is a member of the tyrosine kinase family and we note that tyrosine kinase receptor activation has been linked to the regulation of the sodium ion–proton antiporter²⁵. We also note that the *met* proto-oncogene locus expresses two transcripts which appear to be noncoordinately expressed overlapping transcripts³. The genomic sequences encoding these two transcripts could cover greater than several hundred kilobases. Present genetic data³ suggest that the distance between *met* and the CF locus is less than 5,000 kb.

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Retroviral gag and DNA endonuclease coding sequences in IgE-binding factor gene

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Immunoglobulin-binding factors are known to regulate the synthesis of B-cell-derived immunoglobulin heavy-chain isotypes¹. Cloning and nucleotide sequence determination of complementary DNA encoding rodent IgE-binding factors (IgE-BF) revealed that messenger RNA encodes a glycoprotein of 557 amino acids which is expressed as a precursor of relative molecular mass (M_r) 60,000 (60K) in COS7 monkey cells². We report here that the 3' two-thirds of the IgE-BF coding sequence shows a surprising homology (72%) at the DNA level with coding sequences of the *gag* and *pol* (DNA endonuclease) genes of the Syrian hamster intracisternal A particle (IAP H18)³, an endogenous retrovirus. This marked homology demonstrates that the rodent gene encoding IgE-BF is a hybrid gene which evolved very recently by integrating genes of viral origin, and that the encoded polypeptide comprises three separate domains: an IgE-BF domain and retrovirus-derived *gag* and DNA endonuclease-like domains. This may represent the first report of a cellular gene containing a virus-derived coding sequence.

Figure 1 shows a homology matrix comparison between the nucleotide sequence coding for the rodent IgE-BF and that of

the Syrian hamster IAP genome³. A striking homology with IAP is found for the 3' two-thirds (nucleotides 700-1,770) of the IgE-BF coding sequence, whereas the remaining region (1-699) shares no obvious homology with IAP. In IAP, the homology extends over two regions (nucleotides 1,483-2,140 and 5,364-5,772) which are interrupted by a non-homologous sequence of ~3,200 nucleotides bounded by direct repeats of non-identical but homologous sequences (2,142-2,185 and 5,364-5,406). This non-homologous sequence is completely absent in the IgE-BF coding sequence (Fig. 1b). The direct repeats may result in the deletion of a large region of DNA in the IgE-BF coding sequence. The 5' homologous region of IAP exists within the *gag* coding region, while the 3' block contains part of the DNA endonuclease coding sequence^{3,4}. Alignment (Fig. 2) of the nucleotide sequences reveals 72% homology at the DNA level. Martens *et al.*² noted that the deduced amino-acid sequence of the IgE-BF precursor polypeptide shows local homology with retroviral *pol* gene products in the C-terminal region of 50 amino acids. This high degree of sequence homology indicates unequivocally that the two sequences are evolutionarily related.

These results strongly suggest that the rodent IgE-BF precursor comprises two components of distinct origin: the N-terminal one-third (amino-acid positions 1-202) carries a function unique to IgE-BF while the remaining C-terminal portion consisting of *gag* (203-422) and DNA endonuclease (423-557) related domains was derived from a retrovirus or endogenous retrovirus very recently during evolution. That the IgE-BF coding sequence shows a high degree of nucleotide sequence homology with the rodent IAP, together with the existence of *pol*-related sequences in various retroviruses and transposons across a wide taxonomic distance⁴⁻⁹, strongly suggests that a certain type of retrovirus or endogenous retrovirus was inserted into a cellular DNA region proximal to the primordial IgE-BF gene, and these were later

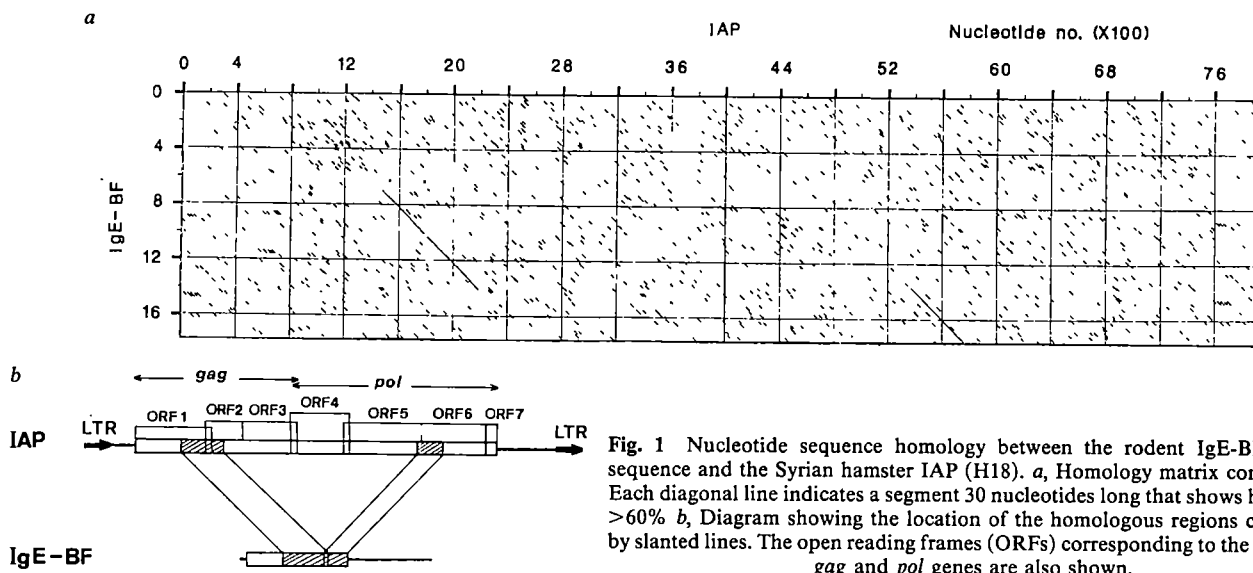


Fig. 1 Nucleotide sequence homology between the rodent IgE-BF coding sequence and the Syrian hamster IAP (H18). a, Homology matrix comparison. Each diagonal line indicates a segment 30 nucleotides long that shows homology >60%. b, Diagram showing the location of the homologous regions connected by slanted lines. The open reading frames (ORFs) corresponding to the retroviral *gag* and *pol* genes are also shown.

A major retroviral core protein related to EPA and TIMP

WE add another curious note to the sequence similarities shared by the proteins identified by biological properties as either the erythroid-potentiating activity (EPA) factor associated with stimulation of erythroid precursors¹ or the tissue inhibitor of metalloproteinases (TIMP). A recent report by Docherty *et al.*² demonstrates that these two proteins have identical sequences.

Using the Intelligenetics PEP program, we have detected regions of similarity between the EPA-TIMP protein and the gag core proteins encoded by retroviruses including human T-cell lymphotropic virus types I, II and III (HTLV-I, -II and -III), bovine leukaemia virus (BLV), Rous sarcoma virus (RSV) and Moloney murine leukaemia virus (Mo-MuLV). These regions are congruent with those conserved among the major retroviral capsid proteins.

These gag proteins are highly divergent among themselves at both the primary and secondary structure levels as predicted by hydrophilicity profiles (Hopps and Woods algorithm)³. Table 1 shows the alignment of the amino-acid sequences, while Fig. 1 shows that the relative position of the regions of similar sequence in the respective proteins is the same. The similarities in sequence and sequence organization may reflect either a common evolutionary origin or common function. It is noteworthy that the only region of sequence similarity between EPA and the human granulocyte-macrophage colony-stimulating factor⁴, besides a short hydrophobic stretch close to the amino terminus, is region III, which is also the most highly conserved region among the retroviral core proteins.

The similarities reported here open the possibility that the major gag core protein may be important not only in viral assembly but also in affecting the cellular physiology so as to favour viral replication



Fig. 1 Regions of similarity between EPA-TIMP and the major retroviral gag core proteins. aa, Amino acids.

acting as a growth factor or a protease inhibitor. In the latter case it could prevent the breakdown of proteins essential for viral replication such as reverse transcriptase or the endonuclease-integrase. These proteins are exposed to the cytoplasmic and nuclear environments during the early stages of the infection process. The major retroviral core protein may also regulate changes in the cytoskeleton during the process of budding. In this regard we note that certain pathogenic determinants of some avian and murine retroviruses have been mapped to the gag-pol region^{5,6}. Using an analogy with the gag core protein function, EPA-TIMP may interact with RNA and with membrane proteins in its regulatory activity.

Many of the transforming oncogenes transduced by retroviruses are synthesized as fusion proteins that contain a substantial portion of the major capsid protein. The deletion of the gag determinants in some cases results in loss of transforming activity¹³ which could be due to loss of growth-promoting or protease inhibitory activities associated with the gag portion of the fusion product.

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Table 1 Alignment of amino-acid sequences in regions of similarity shown in Fig. 1

Region	Origin	Position	Sequence
I	HTLV-III	35	E V I P M F S A L S E G A T P
	HTLV-I	32	P G S P Q F M Q T I R L A V Q
	HTLV-II	32	E G S P Q F M Q T I R L A V Q
	BLV	32	P G S Q V W I Q T L R L A I L
	Mo-MuLV	34	V E P G K L T A L I E S V L I
	RSV	33	I T M A E V E A L M S S P L L
	EPA	-2	P T M A P F E P L A S G T L L
II	HTLV-III	112	Q E Q I G W M . T N N P P I P V G E I
	HTLV-I	112	E Y Q Q L W L . A A F A A L P G S A K
	HTLV-II	112	E T Q N L W L . A A F S T L P G N T R
	BLV	112	Q Y Q N L W L Q A G K I S L L V L Q L
	Mo-MuLV	109	Q L L L A G L Q N A G R S P T N L A K
	RSV	116	Q G Q A A L L R P G E L V A I T A S A
	EPA	75	L G D A A D I R F V Y T P A M E S V C
III	HTLV-III	150	I L D I R Q G P K E P F R D Y V D R F
	HTLV-I	133	W A S I L Q G L E E P Y H A F V E R L
	HTLV-II	133	W A A I L Q G L E E P Y C A F V E R L
	BLV	133	W S T I V Q G P A E S S V E F V N R L
	Mo-MuLV	130	ITQGPN . . I T Q G P N E S P S A F L E R L
	RSV	149	W A D I M Q G P S E S F V D E A N R L
	EPA	113	Q D G L L H I T T C S F V A P W N S L S
IV'	HTLV-III	193	N A N P D C K T I L K A L G
	HTLV-I	185	N A N K E C Q K I L Q A R G
	HTLV-II	185	N A N K E C Q K L L Q A R G
IV	RSV	191	F R Q K S Q P D I Q Q
	EPA	153	F P C L S I P C K L Q

Similarities among the major gag core proteins of HTLV-III⁷, HTLV-I⁸, HTLV-II⁹, BLV¹⁰, Mo-MuLV¹¹ and RSV¹² and EPA¹ or TIMP² and the granulocyte-macrophage colony-stimulating factor⁴ are shown. Common amino acids and conservative substitutions are boxed. A repeat is present in region III in Mo-MuLV.

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Body size and biomass

A RECENT article¹ described several examples of the double logarithmic relationship between the number of individuals and their body length for mixed arthropod populations resident on vegetation of various surface fractal dimensions. Eight examples were given graphically spread over five diagrams. The mean of the slopes of the regression lines through the diagrams can be derived, from the individual slopes given¹, as -3.028 ; that is within 1% of -3 . A slope of -3 indicates a relation of the form: $\log N = \log K - 3 \log L$, where N is the number of individuals, K is constant and L is a body length; that is, $N = KL^{-3}$.

However, if we assume a constant body shape and that all arthropods enjoy a uniform density for a first approximation, we have: $m = k_0 L^3$, where m is body weight and k_0 is a constant. This converts to $L = k_1 m^{1/3}$, where k_1 is a constant. Therefore, $N = K(k_1 m^{1/3})^{-3} = k_2 m^{-1}$ where k_2 is a constant. But $m = M/N$, where M is the total biomass of all N individuals of a given size. Therefore, $N = k_2(M/N)^{-1} = k_2(N/M)$; that is, $M = k_2$. In other words, the biomass of individuals of one size, summed over all species within a community of mixed arthropods, is constant.

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Polarity reversal in the Solomon Islands arc

IN a recent letter, Cooper and Taylor¹ present evidence for two juxtaposed Wadati-Benioff zones in the Solomon Islands, and state that the seismic data provide the first direct evidence of a reversal of subduction polarity at an island arc.

The concept of arc polarity has received considerable attention in the literature²⁻⁴, particularly as such studies suggest how the tectonic settings of former volcanic belts might be interpreted. Cooper and Taylor, following the observations of other researchers on the two seismic zones in the Solomons⁵⁻⁸ raise one of the fundamental tectonic problems for this region, but in doing so fail to give perspective to their proposal by highly selective referencing—ignoring many contributions which might negate or modify their conclusions—and by greatly simplifying the dating of Tertiary volcanism in the region.

Central to the thesis of Cooper and Taylor is the concept that the Ontong Java Plateau collided with the North Solomon Trench sometime during the Miocene. A number of researchers who have been actively working in the Solomons have, over the last decade or so, questioned to varying degrees the concept of collision⁹⁻¹³ and there are strong geological grounds for suggesting that the Solomon Islands may represent the leading edge of the Ontong Java Plateau¹³⁻¹⁵. If two initially quite separate crustal blocks collided, the question rises as to where this collision zone is to be located so that it is in accordance with the known geology.

Arc polarity reversal in the Solomons is often associated with the collision model, following a widely quoted paper by Halunen and Von Herzen¹⁶. Yet what has received far less attention is that one of the major pieces of evidence cited by Halunen and Von Herzen in support of arc polarity reversal (high heat flow on the south-west flank of the Solomons) is more likely associated with the Woodlark Spreading Zone^{17,18}. The presence of an incipient seismic zone dipping south-west below Santa Isabel is interesting, yet its absence to the north along Bougainville requires special pleading by Cooper and Taylor. That this incipient seismic zone is a remnant subduction zone is one of a number of possibilities and until new geological evidence is forthcoming, I agree with Coleman¹⁹ that the concept of north-east-facing arc polarity in the Solomons during the Palaeogene is not proven.

Cooper and Taylor state, as support for their model of collision and polarity reversal, that there was a hiatus in arc volcanism lasting from late-early to early-late Miocene. However, on many of the islands, evidence for the age of volcanism is very poor and no clear age groupings may be given. On Bougainville the Kieta volcanics are dated as Oligocene? to lower Miocene?²⁰, though it is noted that unconsolidated equivalents at Kanga Hill range through to possible Pliocene age. Unnamed volcanics are equated in age with either the older Kieta volcanics or the younger Emperor Range volcanic beds merely on their location and erosional state, while the Buka Formation is dated on geomorphic evidence alone.

In the Shortland Group, volcanism is considered to be pre-Pliocene²¹; Turner and Ridgeway emphasize the lack of fossil and radiometric age control on the various volcanic episodes. It has been suggested that in the New Georgia Group volcanism commenced during the Pliocene¹⁸, though there is evidence for volcanism extending well back into the Miocene^{21,22}. On Choiseul²³ the oldest preserved post-basement sediments were deposited in the late Oligocene to early Miocene and, during early and middle Miocene, these contained increasing amounts of andesitic volcanic material. Possibly the best

evidence for a hiatus in arc volcanism comes from Guadalcanal²⁴ where the top of the Suta volcanics is placed on the Aquitanian-Burdigalian boundary. However, the age of the base of the Gold Ridge volcanics is uncertain and Hackman²⁴ notes that Suta-type volcanism continued intermittently in north-central Guadalcanal into the Pliocene. For San Cristobal, any deduction regarding the tectonic position of the island from Oligocene time onward that is based solely on the absence of arc volcanism in its stratigraphical record, is likely to be incorrect. In complex island arc settings, such as the Solomons, with rapid facies variation, localized unconformities, spasmodic volcanic episodes, and the paucity of age controls on volcanism, it is premature to make emphatic statements about breaks in volcanism and then to use the assertions to lend support to the notion of collision and arc polarity reversal.

The suggestion by Cooper and Taylor that volcanism on Savo may be the product of reactivated magmatism associated with a relic slab is not supported by petrological data¹³. The broad similarities in petrography, mineralogy and geochemistry between lavas from Savo, north Guadalcanal and Vella Lavella suggest, as a working hypothesis, that the same tectonic and magmatic phenomena have been responsible for the style of volcanism in these three localities.

Finally, I draw attention to the warnings of Arculus and Johnson²⁵ and make a plea that tectonic models proposed for island arcs such as the Solomons should balance generalized arc models carefully with the unique features as displayed in the field evidence and field relations of this enigmatic fractured island chain.

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TAYLOR AND COOPER REPLY—Ramsay rightfully asserts that we did not cite many conflicting articles on the geology of the Solomon Islands in the short introduction to our paper¹.

There are two completely opposite interpretations of Solomon Islands geology. Ramsay² and Turner³ champion the interpretation that the Cretaceous oceanic basement on Malaita, Santa Isabel, Choiseul and Guadalcanal is similar, that the post-Eocene sediments on Malaita and Santa Isabel include fine-grained products of volcanism on Choiseul and Guadalcanal, and that an Oligocene arc was built on the leading edge of the Ontong Java Plateau above a north-east-dipping subduction zone. In contrast, Kroenke^{4,5} and Coleman^{6,7} champion the interpretation that whereas the unmetamorphosed basement and pelagic sediments of Malaita and Santa Isabel are similar to those of the Ontong Java Plateau, they are quite different from the metamorphosed basement and volcanoclastic sediments of Choiseul and Guadalcanal, requiring a Miocene collision between the two provinces, involving obduction of the former above a south-west-dipping subduction zone. The first group interpret the *en echelon* fault-bounded, peridotite-gabbro Korighole trend as a structural high forming a sediment barrier between the two provinces, whereas the second group interpret the same feature as the suture between the two provinces. The geology is sufficiently complicated that some authors including Coleman⁶⁻⁸ and Hughes and Turner^{3,9} have published self-contradictory interpretations. Thus, the evidence from Solomon Islands geology alone for a north-east-facing middle Tertiary arc which collided with the Ontong Java Plateau in the Miocene is ambiguous⁸ and open to conflicting interpretations^{2,3,5,7}.

Other evidence presented by Halunen and Von Herzen¹⁰ in support of arc polarity reversal includes the high heat flow and high shear-wave attenuation on the Solomon Sea side of the arc, the location of active volcanoes on the south-west side of the islands very close to the New Britain-San Cristobal trench, and the presence of deep (>400 km) earthquakes beneath the islands. Taylor and others^{8,11,12} have already noted that most of these phenomena are the result of active seafloor spreading in the Woodlark Basin and subduction of the spreading ridge

beneath the island arc. The deep earthquakes can be related to present subduction beneath the north-west Solomons and to either present north-east or past south-west subduction beneath the south-east Solomons¹³.

Ramsay also points out the lack of radiometric age dates on igneous rocks in the Solomon Islands and the fact that most of the volcanic formations are dated by stratigraphic and fossil evidence, often with poor control. Nevertheless, the early and middle Miocene is a rather well-constrained period in which the widespread deposition of limestones, calcarenites and foraminiferous marls (the Hautara, Mbetilonga and Mbonehe, and Keriata limestones on San Cristobal, Guadalcanal and Bougainville, respectively) mark a regional gap in the volcanic record^{5,6}. There are locally intercalated volcanoclastic sequences, and extensive greywackes on Choiseul and Guadalcanal, but these appear to be derived from erosion of earlier volcanics^{5,6,14}. There are no dates older than 7 Myr on igneous rocks associated with the present north-east subduction⁵. Although we noted that the apparent middle Miocene hiatus in volcanism may be evidence of a temporary cessation of subduction during the polarity reversal¹, we did not emphasize this point because the evidence from the Philippines and other areas indicates that volcanism and even double-sided subduction may occur during an arc polarity reversal.

What then is the remaining evidence for arc polarity reversal in the Solomon Islands? The term 'arc polarity reversal' was coined by Karig and Mammerickx¹⁵, who were the first to propose that this process had occurred in the Solomon Islands, primarily on the basis of studies of the New Hebrides along-strike. They assumed that all marginal basins were formed by crustal extension behind trenches, and therefore that the New Hebrides and Solomon Islands arcs formerly faced north-east. Although this assumption has since been proven incorrect^{16,17}, it is still true that the geology and geophysics of the island arcs along-strike from the central Solomons provide the least controversial evidence for arc polarity reversal. Petrochemical, sedimentological, palaeomagnetic and radiometric age data from the New Hebrides and Fiji all confirm a north-east-facing Vitiaz arc in the late Oligocene-early Miocene¹⁸⁻²¹. Vitiaz arc volcanism ceased in the late-early Miocene, the area was rifted in the middle Miocene, and following arc polarity reversal at ~10 Myr BP, a new arc was built, beginning at 7 Myr BP¹⁸⁻²¹. The Vitiaz trench and adjacent north-east edge of the Fiji Plateau still preserve the classical trench/fore-arc morphology/structure of the former north-east-facing arc²²; the same is true of the Kilinailau-Manus trench and the remnant forearcs north and east of Bougainville, New Ireland and Manus, on the other side of the

central Solomon Islands^{23,24}. Therefore, although some arguments used by early authors^{10,15} in favour of arc reversal have since been disproven, reversal of the island arcs adjacent to the central Solomons remains well documented by both island geology and marine geophysical evidence. An independent mid-Tertiary evolution of the central Solomons on the Pacific Plate would require over 1,000 km of differential movement with respect to the surrounding arcs with which they have long been associated⁶.

Given this context, we reaffirm our previous conclusion that the two juxtaposed Wadati-Benioff zones of opposite polarity provide direct seismic evidence of a reversal in subduction polarity at the Solomons island arc¹. Furthermore, although intermediate-depth seismicity on the south-dipping remnant subduction zone is only observed adjacent to the young Woodlark Basin, significant shallow seismicity is associated with this feature to the north-west^{1,25}. Finally, the location of recent volcanism on Savo, 100-150 km above the relic slab, surely implies some genetic relation between the two.

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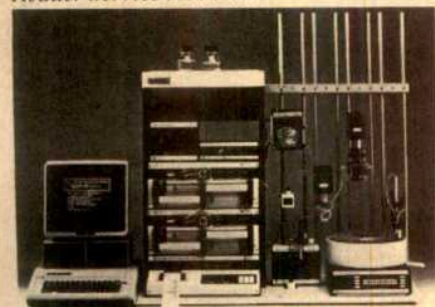
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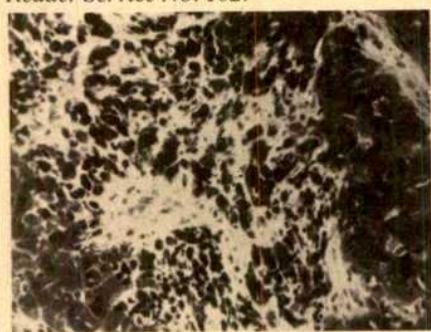
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The indium-III labelled Myoscint there-
fore binds exclusively to necrotic cardiac
cells and images of these are obtained us-
ing a conventional gamma camera.

Reader Service No. 108.

• Serotec of Bicester, Oxfordshire, has
announced an extended range of con-
jugated monoclonal antibodies. The com-
pany developed a method of conjugation
which makes it possible to add labels to
most antibodies in the Serotec range of
monoclonal products. These labels in-
clude FITC, TRITC, peroxidase and alka-
line phosphatase. The conjugates are sup-
plied in 1 ml vials, with a concentration of
bound antibody of 1 mg ml⁻¹.

Reader Service No. 109.

• Hybritech's list of monoclonal anti-
bodies has been expanded to include
murine monoclonal antibody to human
calcitonin, used in studies of certain carci-
nomas and bone disease, and monoclonal
antibody to bombesin, a tetradeca-
peptide, present in mammalian tissues
including brain and gut. Also available
now is pooled AE1 and AE3 monoclonal
antibodies to human epithelial keratin,
which should prove useful for the positive
identification of normal and abnormal
epithelial cells in tissue sections and cul-
ture, and for distinguishing carcinomas
from non-carcinomas. And monoclonal
antibodies to hepatitis B surface antigen
and subtypes as, adw and ay are also avail-
able, which should help in a new and im-
proved methods for hepatitis screening
and diagnosis.

Reader Service No. 110.

• A new HistScan monoclonal detector
kit for the localization of both mouse and
rat monoclonal antibodies following their
reaction with tissue antigens is now avail-
able from Biomed. This monoclonal de-
tector kit is designed to simplify the tissue
immunostaining technique using mono-
clonal antibodies specific for tissue-
associated antigens. It is based on the new
avidin biotin complex technology kits con-
tain all reagents needed to detect mono-
clonal antibodies in 150 tissue sections.
Also contained in each kit are haematoxy-
lin counter-staining reagent, concentrated
chromogen reagents and pertinent block-
ing solutions to minimize nonspecific
staining. The reagents forming the kit are
supplied in a ready-to-use form together
with a step-by-step procedure outline and
other technical information. When stored
at 4°C, the kit has a shelf life of one year.

Reader Service No. 111.

• A new edition of the Boehringer Man-
nheim Biochemicals Catalogue is now
available. It lists over 1,500 products in-
cluding products for molecular biology,
immunology, cell culture and general
biochemistry. This revised edition is de-
signed to be easy to use. The main section
is an alphabetical product listing which
contains all the pertinent information on
product specifications. In addition, there
are quick reference listings for molecular
products and for immunology and cell-
culture products, extensive cross refer-
ences and indexes organized both
alphabetically and by product group. For
a free copy contact Boehringer Mannheim
at 7941 Castleway Drive, P.O. Box 50816,

Indianapolis, Indiana 46250.
Reader Service No. 112.



Tago's new reagents for immunohistology.

• Tago's series of horseradish
peroxidase-antiperoxidase (PAP) soluble
complexes has been specially prepared for
use in immunohistological staining and
quantitative enzyme immunoassays.
These convenient, step-saving reagents
provide a more sensitive assay than
fluorochrome-conjugated antibodies and
a higher signal than standard peroxidase-
conjugated antibody. They also may be
used with fixed tissues as well as with
frozen sections in detecting most antigens.
PAP complexes are now available for ap-
plications with goat or rabbit primary im-
munoglobulins. They are effective at
working dilutions of 1:20,000 for ELISA
and up to 1:200 for tissue staining proce-
dures. PAP complexes can be linked with
primary antibodies through second anti-
bodies to goat or rabbit IgG.

Reader Service No. 113.

• Dako Corporation is introducing con-
trol slides in 10-packs — one stained for
reference and nine unstained slides. These
ready-prepared controls can reduce time
spent searching for, acquiring and proces-
sing tissue. Hard-to-obtain controls for
antigens such as α -fetoprotein, papillo-
mavirus, herpes simplex virus and nearly
20 other immunohistochemical markers
are included in this new product line. Each
slide is ready for immediate use. Each kit
includes recommended step-by-step proce-
dure and a technical staining description.

Reader Service No. 114.

• Adding to its range of arachidonic acid
metabolites, Seragen has introduced a
leukotriene B₄ ³H RIA kit. With its highly
specific sensitivity, the researcher can
achieve accurate results with samples as
low as 10 pg per 100 μ l in a simple 2-hour
room temperature assay. The antiserum
has low cross-reaction with other leuko-
trienes and HETEs so that no HPLC sam-
ple separation is necessary.

Reader Service No. 115.

• Coulter Immunology has announced
the availability of three exclusive leuko-
cyte monoclonal antibodies. The new
Coulter Clone monoclonal antibodies —
2H4-RD1, 4B4-RD1 and Ta1-RD1 — will
enable investigators to identify functional
T4 subsets which play precise roles in the
onset of autoimmune and immune de-
ficiency diseases. Anti-2H4-RD1 defines
the functionally unique T4+ subset, sup-
pressor inducer, which falls dramatically
in certain patients with the onset of syste-
mic lupus erythematosus. 4B4-RD1 de-
fines the T4+ cell subset, helper inducer,
and may be the key parameter for the

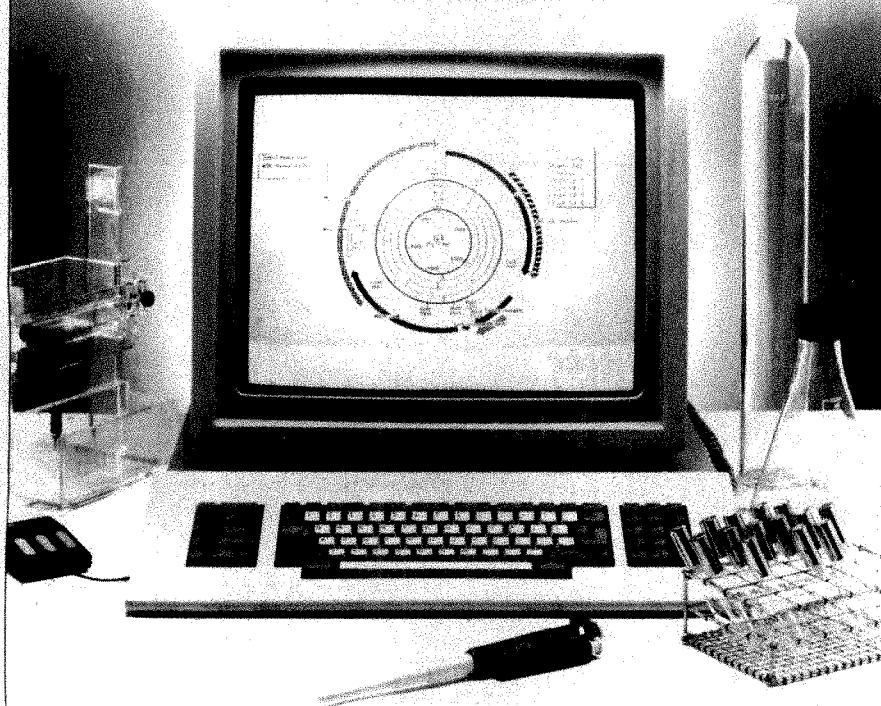
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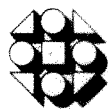
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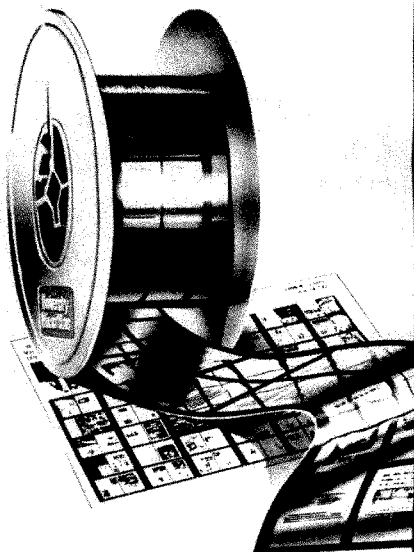
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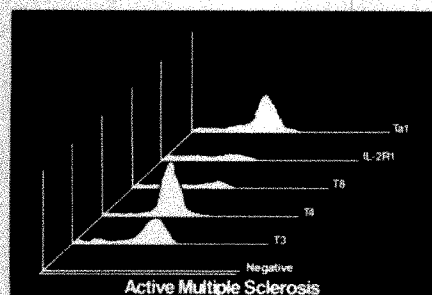
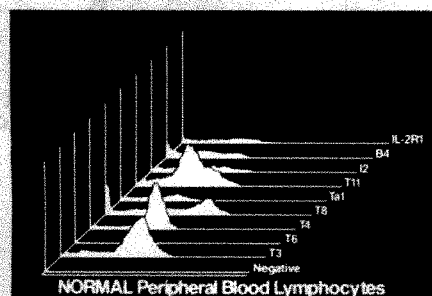
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evaluation of immune deficiencies such as AIDS. These major subsets can be identified by simultaneous analysis with Coulter Clone T4-FITC. T4 is a cell-specific activation antibody available only from Coulter. A recent study of multiple sclerosis patients demonstrated positive correlation between increased T4+ cells in the peripheral blood and systemic immune activation in the disease.

Reader Service No. 116.

• A new Pentex antigen/antibody enhancement medium and antisera diluent called Mod-U-Cyte is now available for research and manufacturing use at two protein concentrations, 6% and 20%. The suppliers, Miles Scientific, say its advantages include: Rh and certain other rare antibodies are more reactive at both pre- and post-antiglobulin test phases when compared to conventional, highly polymerized and low ionic strength bovine albumin solutions; certain weak clinically significant IgM antibodies can be detected without using proteolytic enzyme-premodified red blood cells; and with Mod-U-Cyte as a blood grouping and typing antisera diluent, most titres are extended two to 16 times when compared to solutions using bovine albumin as a diluent. Mod-U-Cyte may enhance availability of cell surface antigen sites.

Reader Service No. 117.

• Jackson ImmunoResearch has introduced a new line of over 200 biotinylated antibodies which have a 13-atom spacer between biotin and the antibody. This results in a significant increase in sensitivity. The spacer appears to extend the biotin moiety far enough from the antibody surface to make it more accessible to biotin-binding sites on streptavidin and avidin, especially when they are labelled with high molecular weight enzymes. It appears that either more enzyme-avidin conjugate can be spatially accommodated on the antibody surface or perhaps the extended biotin has better access to its

binding sites which are sterically recessed from the surface of the enzyme-covered avidin. The new products, designated Biotin-SP to indicate biotin with a spacer, will be available on all biotinylated AffiniPure antibodies and ChromPure proteins. Biotin-SP enzymes containing the same 13-atom spacer will also be available to form avidin-biotin-enzyme complexes for use in histochemical/cytochemical procedures requiring ultra-high sensitivity. Fluorophore-labelled and enzyme-labelled streptavidin and avidin are available for use with Biotin-SP-AffiniPure antibodies and Biotin-SP-ChromPure proteins.

Reader Service No. 118.

• Celltech Limited produces a range of immunoprocessing reagents based on the company's expertise in large scale monoclonal antibody production. In September 1985 Celltech extended its range of monoclonal-antibody-based immunoprocessing reagents with the introduction of Resolute IL-2 and IL-2 IRMA, for the purification and assay, respectively, of human interleukin-2. The reagent gives a high purity of interleukin-2 in a single-step chromatographic process, and is a reliable, rapid and reproducible alternative to the more traditional 'bioassay' in-2. Celltech also supplies monoclonals for ABO blood typing.

Reader Service No. 119.

• Euroclone is a new European company specializing in the contract production of mammalian secreted products such as monoclonal antibodies, human growth hormone and tissue plasminogen activator. The company is interested in both small and large scale production orders. Production is based on the computerized facility, Acusyst-P. This system, manufactured by Endotronics Inc., uses hollow-fibre technology in combination with a dosage of cell media. For each customer all the manufacturing conditions are very well defined and reproducible. The cost per gram of secreted product should be significantly lower than the usual level. Euroclone is a joint venture of Dalton-Waalwijk, of the Netherlands, and a well-known European immunology institute.

Reader Service No. 120.

• BioTope, Inc. of Bellevue, Washington has announced two new immunoassay kits for the cancer research market. These assay kits are claimed to be the first commercially available immunoassays for detecting and measuring human transforming growth factor alpha (hTGF- α) and human epidermal growth factor (hEGF). The kits detect these growth factors in urine. Based on BioTope's proprietary microassay technology, BioMAT, the kits combine speed, economy and reproducibility. BioMAT should find numerous applications in immunoassays, receptor assays and DNA probe assays.

Reader Service No. 121.

• A radioimmunoassay kit suitable for the determination of the level of atrial

natriuretic polypeptide (ANP) in unextracted plasma samples has been developed by Research & Diagnostic Antibodies. ANP is the recently described heart hormone involved in the regulation of blood pressure due to its hypotensive, diuretic, and natriuretic actions. The assay can accurately determine the quantity of ANP in 0.05 ml to 0.10 ml of unextracted normal plasma. The assay has a minimum sensitivity of 2 pg ANP per tube.

Reader Service No. 122.

• The Ribi Adjuvant System (RAS) from Ribi ImmunoChem Research, Inc. is an easy-to-prepare adjuvant system intended as an alternative to the classical Freund's water-in-oil emulsion. RAS is a stable oil-in-water emulsion. Adjuvanticity is improved when the adjuvant is incorporated together with the antigen into the oil phase, rather than incorporating the antigen into the aqueous phase. The mixture is then dispersed in a large volume of saline containing microscopic oil droplets. This makes it possible to have a reduced oil concentration (2% instead of 50%), thereby reducing emulsion viscosity. In RAS the tubercle bacilli contained in complete Freund's adjuvant are replaced by trehalose dimycolate, a highly purified adjuvant. Monophosphoryl lipid A (detoxified endotoxin), *Salmonella typhimurium* mitogen or cell wall skeleton can be added to further enhance immune response.

Reader Service No. 123.

• Seven monoclonal antibodies specific for cytokeratin intermediate filament proteins are now available from Amersham International. This range includes antibodies for use with formalin fixed paraffin embedded material, having an expected application in the sub-typing of normal and pathological epithelia. Also included are antibodies recommended for use with unfixed frozen material. All these antibodies can be used for immunofluorescence staining of cultured cells and 'Western' blot analysis of cytokeratin proteins.

Reader Service No. 139.

• Gibco/BRL's range of products for immunology consists of monoclonal antibodies, hybridoma screening kits and reagents for immunoprecipitation and purification of immunoglobulins. Products for immunodetection based on streptavidin-biotin technology include biotinylated secondary antibodies, streptavidin conjugates and enzyme substrates designed for use in the detection of target antigens by various immunoassay procedures such as ELISA, immunoblotting and immunocytochemistry. For immunoprecipitation BRL offers streptavidin agarose, protein A agarose and immunoprecipitin. For further information on all Gibco/BRL streptavidin-related products use the reader service code to send for a brochure illustrating the applications of streptavidin in the detection of protein, cells and DNA.

Reader Service No. 124.

- Bio-Rad has produced a new publication giving details of a range of high-quality instruments, systems and reagents for purifying and characterizing monoclonal antibodies. The booklet provides information on techniques for purifying antigen by gel electrophoresis and chromatography, screening hybridomas and characterizing hybridoma-secreted immunoglobulins. In addition, there are descriptions of HPLC purification systems and applications for monoclonal antibodies. *Reader Service No. 125.*
- ICN Biochemicals announces the availability of Lipid A, a biologically and immunologically active toxic component of lipopolysaccharides. It has been isolated separately from the serologically specific polysaccharide fraction. Lipid A is known to elicit endotoxic responses, and the availability of this purified form should make it easier to study those responses without the problem of interference from the polysaccharide fraction of lipopolysaccharide. *Reader Service No. 126.*
- Eight new affinity purified antibodies (APAs) to human IgG (Fc), human IgG (Fab) and their corresponding conjugates (FITC, peroxidase, alkaline phosphatase) have been added to Bio-Yeda's range. Products in this series comprise APA to mouse IgG (Fc) and mouse IgG (Fab) and conjugates, and APA to rabbit IgG (H+L). Bio-Yeda APAs and conjugates are purified from goat serum by affinity chromatography with immobilized antigens. At least 95 per cent of the eluted antibodies are active as determined by use of quantitative immunoprecipitation analyses. Because of minimal interspecies crossreactivities to mouse and rat serum proteins, the APAs to human Fab and Fc and their respective conjugates may be used for screening of human monoclonal antibodies produced by hybridoma cells grown *in vitro*. The affinity purified anti-human Fab reagents offer the advantage of increased sensitivity for all the possible human immunoglobulin isotypes (IgG all subclasses, IgA, IgM, IgD, IgE) in biological fluids of tissues in normal or pathological situations. APAs to human Fc and respective conjugates detect specifically human IgG with its four subclasses IgG₁, IgG₂, IgG₃ and IgG₄. The conjugates of these anti-human immunoglobulin reagents are very effective as secondary reagents in ELISA, immunoblotting and immunohistology. *Reader Service No. 127.*
- RIA (UK) Ltd has introduced the Histoclone range of immunohistochemistry kits, based on a series of eight monoclonal antibodies. Designed for the specific identification of human antigens, the kits use routinely fixed, paraffin-embedded tissue samples, or frozen tissue sections. The monoclonal antibodies have been raised against eight diagnostically crucial antigens: luteinizing hormone, human chorionic gonadotropin, growth hor-

none, thyroid stimulating hormone, prolactin, alpha-fetoprotein, carcinoembryonic antigen and human B cells/Ia-like. *Reader Service No. 128.*

- New from Biotest-Serum-Institut GmbH is a monoclonal antibody against interleukin-2 receptor (Clonab IL-2 R) which detects human activated proliferating T cells. Extremely high purity allows 10 functional tests and more than 1,000 immunofluorescence tests from 1 ml of lyophilized preparation (1 µg of highly purified antibody). *Reader Service No. 129.*

- Becton Dickinson has introduced a new second-antibody for immunofluorescence staining. The monoclonal rat anti-mouse kappa is conjugated to phycoerythrin as a bright second-step reagent for indirect analysis. It is significantly brighter than a FITC conjugated second-step, when used in flow cytometry or microscopy. Anti-mouse kappa phycoerythrin detects low-density antigens where resolution between stained and unstained cells is critical. *Reader Service No. 130.*

- Costar's new Transtar-96 is a portable 96-well liquid handling system with an adjustable volume range of 25–200 µl. The system is completely autoclavable for use in a sterile environment. The heart of the system is a polystyrene radiation-sterilized disposable unit with 96 pipette tips moulded in one piece, covered by a medical-grade silicone rubber membrane. Applications include changing cell culture medium and liquid transfer for assays, for instance in screening of monoclonal antibodies. The 96-well microplate with low evaporation lid minimizes overall evaporation and eliminates "edge effect". *Reader Service No. 131.*

- Metachem Diagnostics' range of specialist antisera for immunocytochemistry now includes antisera to corticotropin releasing factor, growth hormone releasing factor, PHM-27, calcitonin gene related peptide, neuropeptide Y, galanin and peptide YY. The antisera are lyophilized and sealed under nitrogen. After reconstitution the antiserum is ready for use for either PAP or FITC techniques. Supplied with each antiserum is a data sheet with recommended immunohistochemical protocol. *Reader Service No. 132.*

- Cambridge Research Biochemicals have extended their range of peptide antisera to include polyclonal antisera raised to human neurone specific enolase for radioimmunoassay. Pure human neurone-specific enolase is also available. *Reader Service No. 133.*

- The *ras* family of oncogenes are the oncogenes most commonly found in human tumours and have been associated with bladder, lung and colon carcinomas, and various leukaemias. Because the proteins produced from *ras* oncogenes have molecular weights of 21,000, they are referred to as "p21" proteins. The Cetus *ras*

oncogene product monoclonal antibody can be used for experiments designed to explore *ras* oncogene expression in tumours and mutant-specific polyclonal antibodies can be used to detect *ras* mutations, resulting in single amino acid changes in p21. These single amino acid mutations activate the *ras* proto-oncogene to the oncogene form. Rabbit polyclonal antibodies capable of detecting oncogene forms of p21 with serine, valine, arginine or aspartate at position 12, and anti-p21 pan-reactive monoclonal and polyclonal antibodies were introduced at the *Nature* Conference in San Francisco last month. *Reader Service No. 134.*

- The latest Immunochemical Products catalogue from Zymed includes a long list of affinity purified antibodies to mouse, human and rat immunoglobulins. Each antibody is characterized according to its reactivity to a specific class of immunoglobulins and cross-species reactivity. The streptavidin/biotin system is of particular interest to those who need to enhance the signal for enzyme immunoassay, histochemical and cytochemical staining whilst retaining negligible background. Monoclonal antibodies are also listed as are recommended methods used in immunology. *Reader Service No. 135.*

- Organon Teknika has introduced what is claimed to be the first non-isotopic immunoassay for FSH and LH in serum or plasma. Both tests, FSH-Nostika and LH-Nostika, are based on the sandwich principle for the detection of physiological values of the respective hormones. Incubations are at room temperature, and results are available on the same day (total incubation of 4½ hours). The system gives good reproducibility and has the same sensitivity as radioimmunoassays. *Reader Service No. 136.*

- The new disposable affinity columns from Biological Consultancy Services (BCS) make it possible to purify mouse or human IgG, IgM, IgA from serum, tissue culture fluid or ascites in one step. Each column can be used several hundred times: thus they will purify immunoglobulin from as little as 1 ml fluid or as much as several litres. Immunoglobulin can be purified to >99% purity in one step. There is no need to concentrate the starting fluid or to partially purify by ion-exchange or molecular size chromatography. *Reader Service No. 137.*

- Labsystems' Uniskan range of photometers includes the new Uniskan II, designed for use in absorbent and turbidimetric measurements typical of enzyme immunoassay and microbiological work. Uniskan's optics are based on vertical photometry which eliminates the adverse effects of layering, evaporation and other sources of error. The life of the light source has been extended to a quarter of a million measurements by means of pulsing the beam. *Reader Service No. 138.*

Last high-noon for UNESCO

The British government seems determined to pull out of UNESCO. Here is a last-minute plea that it should change its mind. But UNESCO's problems will remain.

THE fine Corbusier building in Paris inhabited by UNESCO (United Nations Educational, Scientific and Cultural Organization) is not noticeably upset by the prospect that the United Kingdom may cease to be a member at the end of the year (three weeks from now). After all, the least successful of the UN's agencies has just returned from a conference at Sofia at which it seemed to make concessions to its critics, and gained the impression that some of them had been silenced. After the departure of the United States (responsible for 30 per cent of the 1984 budget), which seems to have left most things unchanged, how can the defection of the United Kingdom (with its mere 7 per cent) shake the edifice substantially? The simple answer is that the present time is a lull before a storm. UNESCO is going to have to change, not merely because of the complaints against it but from necessity. The strongest argument why the British government should not now pull out is that, if it stays, it has a good chance of being showered with the plaudits that will be showered on the reformers.

A still better reason for staying in is that there is much to do. UNESCO's errors are mostly errors of commission. It has tilted at impossible windmills, such as the objective of bringing about a new world information order (a device for making sure that governments are never offended by what their newspapers publish). Yet UNESCO has also done good works, it remains, for example, the only substantial source of support for the International Council of Scientific Unions. By any standards, a programme composed of such a mixture of the impossibly utopian and the practically utilitarian cannot but be endearing. The challenge, and the task that will remain even if Britain leaves, is to hammer this programme into shape.

For all its outward toughness about UNESCO, even the British government has not faced the central error in UNESCO's way of doing business, the generally accepted belief that the organization is meant to play a decisive part in the development of developing countries. Mr Timothy Raison, the British Foreign Office minister responsible for overseas aid (and for UNESCO), who has been sympathetically hard-headed on British membership over the past year, fell into the familiar trap in his speech during the House of Commons debate on UNESCO last month. Get those civil servants out of Paris, and into the Field, is the cry. The reality is that there are half a dozen other UN agencies whose terms of reference enjoin them explicitly to assist development, and whose budgets are more nearly commensurate with the task. A large part of UNESCO's difficulty over the past few years is that the majority of its members (developing countries) have insisted that it should become another aid agency, and that its paymasters (governments like the British) have weakly fallen in with the proposal.

By all accounts, the same error was repeated at last week's meeting of the British National Commission for UNESCO, the representative body of the great and the good which the government feels compelled to consult (but not compelled to listen to) at times like these. Most voices spoke for UNESCO's further transformation into a lowly version of, say, the Rockefeller Foundation that would remain hamstrung by its constitution, and by the need to deal equally with all of its constituents. The need for development, as this year's experience in most of Africa has all too plainly shown, is too urgent to be safely

neglected for much longer. But that, unfortunately, does not imply that UNESCO, already driven far off course, should be thrown ineffectually into the battle. The scale of its operations is simply too small to make much difference. But equally, it is not necessary that UNESCO should continue indefinitely to be the chief source of support for worthy organizations such as the International Council of Scientific Unions, which could (and should) keep itself alive by means of contributions from its members. UNESCO needs most of all to discover what it is for, and to pursue those goals wholeheartedly.

What might be done? It is not so long since UNESCO claimed to be an important influence in the teaching of science and technology in schools. It retains some influence in that field, spending modest sums on useful good works such as the design and manufacture of school science equipment, notably through its centre at the University of Delhi. But UNESCO has never had the nous to appreciate the generality of the difficulties of science teaching, the simple truth that rich countries such as the United States are as perplexed as the poorest among the UN's members. That is a field that a reformed UNESCO would plough. And why not extend the same principles both to other parts of the curriculum of the young?

In short, UNESCO's programmes should be devised to span problems within its terms of reference (education, science and culture) which are common to its members, not the exclusive concern of the poorest among them (or of any other class). Among present programmes, that to catalogue and, if possible, to preserve sites of archaeological or cultural importance to the international community is a good model for what might be done (if a poor model for administration). In the past, UNESCO has similarly helped to reach compromises between the rich and poor countries on questions such as copyright, where the trick has been to reconcile conflicting interests. There are many other issues of this kind that need attention, but which will not be attended to if too many UNESCO members quit.

That is the prize the British government should stay to help to achieve. There are few who would not accept its view that UNESCO as it stands is a badly administered can of worms which, by its reputation, does more harm than good to the cause of international cooperation. The obvious difficulty is that, if UNESCO should now collapse, it would not be reinvented within the lifetime of anybody now alive. Yet the tasks that cry out to be tackled are too important to wait that long, while the shocks to UNESCO of the past two years provide an opportunity that will not soon recur. □

Changing of the guard

The Royal Society has a new president, but it may also need a new policy.

SIR Andrew Huxley, who retired last weekend as president of the Royal Society, has predictably done a splendid job during the past five years. Undemonstratively temperamental, almost the opposite of a power-seeker, his capacity (and liking) for intellectual work has enabled Britain's best known society to enhance its reputation for giving advice that cannot be ignored. During his spell in office, Sir Andrew has also emerged (again

predictably) as a courageous president: he has told off his opposite number in Moscow for the Soviet treatment of Sakharov and, more recently (see p.399), the organizers of next year's archaeological congress at Southampton for their indifference to the principles of freedom in science. That the administration of the society has been refreshingly improved during his tenure of office is an unexpected bonus. He is affectionately forgiven for his intervention on behalf of the British Museum (Natural History) — which should find itself a better name — in its dispute with *Nature* over the public presentation of evolution (see *Nature* 291, 373; 1981).

Huxley's successor, Sir George Porter, will almost certainly do as well. His record in research is similarly distinguished (with a Nobel Prize, while they last, a qualification for the job among other things), but he also has a foot in another camp, the popularization of science. Especially as director of the Royal Institution (Humphry Davy's home for Michael Faraday), Porter has done more than most working professionals to make sure that the world at large should have a better understanding of why science is important. It is no surprise that he should have said at the weekend that, for the next two or three years, the public understanding of science will be one of the Royal Society's preoccupations. There is a great deal of enlightenment to be effected. Everybody will hope that the new president can work a little wonder.

The obvious difficulty is that this is only part of the battle that needs to be fought. Logic would suggest that if the British electorate were better informed about the excitement of what happens in laboratories, it would sense the potential economic as well as cultural value of what is called research and instruct their elected representatives accordingly; and the consequence of that, the argument goes, is that British governments would no longer be able to deal with budgets for research as if they were any old kind of public spending. Unfortunately, in this as in other fields, logic is an inadequate guarantor of good sense, and is in any case slow to work its way through the electoral system. And although most British scientists will now say that their troubles stem from the cuts which there have been in their budgets, that is only half the truth. It matters at least as much that the system of research organizations spending what funds there are has been slow to accommodate itself to the changing environment. The simple consequence has been that potentially creative people have too often allowed themselves to be persuaded that they cannot hope to accomplish much.

In these depressing circumstances, the most urgent need of the British research enterprise is for leadership. It will help a great deal if researchers discover the Royal Society to be hard at work instructing people at large that science is a worthwhile enterprise, but there is more that needs doing. In particular, there is a need that some organization that stands for science, but which also understands the government's need to spend less, should more openly function as an informed critic of mismanagement of the kind that has marked the past few years. Who better than the Royal Society?

There are three stock explanations, one of which is that the Royal Society depends on public funds for most of its support, another that the society delivers its opinions confidentially, to those (in the government and elsewhere) who must act on them and, third, that a society composed of individuals elected for their academic distinction cannot be expected to have what might be called a corporate view. There is something in each of these arguments. It is a particular embarrassment that the Royal Society should both be dependent on the Advisory Board for the Research Councils for its own funds and an *ex officio* member of the same committee, no doubt because of the potential value of its candour. The view that the giving of advice through private channels inhibits public criticism is less cogent. And while it would probably be impossible to win an agreed view by the Royal Society's members on, say, the best way to finance the British Broadcasting Corporation or even the wisdom of replacing *Polaris* by Trident submarines, there is every chance that they would unite behind a measured and public criticism of

government policy towards British science in the past five (or even fifteen) years. What seems not to be understood is the degree to which morale in the laboratories would be stiffened by the emergence of an influential monitor of the present course of black events. It is possible that Sir George Porter has just such a plan hidden behind the slogan of public understanding. Now, at least, the Royal Society has a policy unit that could be the source of the analysis needed to sustain such a role. Why not take it up? There is much to be gained and, now, not much to be lost. □

Broadcasting in chaos

The British government has encouraged a sense of being mean that now extends to the BBC.

LIKE public policy on strategic arms, public policy on broadcasting is also an issue with which the technical community must grapple. The simplest reason is simply given; without the technology, there would be no issue, so that the innovators have a continuing responsibility. The more complicated but more interesting reason is that the innovators (or the inheritors) have a proper self-interest in the use made of the technology; will it, they may ask, further our interest that there may be more discovery and innovation? Those who may be sympathetic to this notion should pay some attention to what is happening to the British Broadcasting Corporation (BBC), certainly the best broadcasting organization in the world.

The BBC is constitutionally a public corporation, legally given (by charter) the right to function as if it were a corporation (or joint-stock company, as the Victorians would have said) but also saddled with the responsibility to broadcast material that gives neither personal nor political offence. But unlike other corporations, the BBC raises the funds it needs to keep on the air by means of an annual payment by those who own television and radio sets. This arrangement, conceived of in the 1920s, when television had not been invented, and when even the ownership of a radio receiver was a privilege, persists to the embarrassment of both the BBC (which needs the money) and the British government (which must periodically sanction an increase in the licence fee as, grudgingly, it did earlier this year).

So should not the market play a more prominent part in matching public expectations of broadcasting organizations to what the broadcasters provide? Especially because it has not escaped public attention that most broadcasting organizations are financed on a commercial basis, selling part of the time for which their audiences are prepared to listen to outsiders with other messages to impart, should not the BBC also sell time to advertisers? It would be unjust to the present British government to complain that it has insisted that this question should be answered. It has merely created the climate in which the question cannot be avoided.

The outcome is a committee under Professor Alan Peacock now brooding on the future financing of the BBC. Last week, the committee organized a meeting at which people whose views are well known repeated them for a gathering of others whose views are equally well known. (There were journalists in attendance, in the gallery.) The most radical (and the best) of the free-market solutions to the government's problem about the BBC (due to Mr Peter Jay, once British ambassador in Washington) is that people should pay for what they receive from among what is broadcast as they do for telephone calls. The trouble is that that is also politically unworkable. Most voters who now say that they would prefer that the BBC should take advertising than that the licence fee should be increased might turn nasty if they thought they would have to pay by the minute not the year. Yet the BBC must somehow be taken off the backs of successive British governments, all of which have been prepared to take credit for its quality while resenting the need to approve new licence fees. Why not turn the corporation into a public foundation which, unlike the Public Broadcasting Service in the United States, would be financed by an endowment, not an annual subvention? □

Apartheid in science

Crunch still ahead for archaeology congress

THE planners of next year's World Archaeological Congress at Southampton (England) have during the past week encountered mounting but conflicting pressures over the exclusion of South African scientists. The most ominous development is the decision of the International Union of Prehistoric and Protohistoric Sciences (IUPPS) to hold a special meeting of its executive committee (in Paris on 17 January 1986) to consider the matter. Meanwhile, the presidents of the Royal Society and the British Academy have spoken out against the exclusion, but the British committee organizing the congress has reaffirmed its earlier decision to ban South Africans.

The meeting of the executive committee of IUPPS (not to be confused with the British committee responsible for the congress) could affect what happens next year in Southampton. In principle, the committee could decide to withdraw its *imprimatur* from the congress. In that case, according to Professor John Evans, the president of IUPPS who is also chairman of the British committee, it would be necessary to "consider again" the arrangements for next year's congress.

The intervention of the presidents of the Royal Society and of the British Academy first took the form of a letter to *The Times*. On 17 November, Sir Andrew Huxley and Sir Randolph Quirk, speaking for their respective academies, referred to their "profound concern" at the decision to disinvite scientists from South Africa. They said that "it is an indispensable condition of holding an international conference that *bona-fide* scholars should be admitted irrespective of nationality, domicile or politics" and went on to describe the decision not to allow South Africans as "deplorable".

Huxley and Quirk went on to say that the decision could mean that Britain would cease to be regarded by bodies such as the International Council of Scientific Unions as "a fit place in which to hold an international scientific congress". Huxley expanded on this theme in his final anniversary address to the Royal Society last Saturday (30 November).

The meeting of the British National Committee on 20 November seems to have surprised those present by the weight of opinion in favour of continuing the ban. On one account, only one of a score of members spoke in favour of rescinding the ban. There are reports of overseas scientists having withdrawn from the congress, but the numbers are said to be "only about thirty". The possibility of moving the congress to another venue had been con-

sidered, only to be discounted on the grounds of lack of time.

At Southampton, the organizers have a sense of having been misunderstood, even misrepresented. Professor Peter Ucko, the secretary of the organizing committee, says that nobody has appreciated the trouble that had been taken to ensure the attendance of genuine scientists from developing countries. In this respect, he says, there is every prospect that the congress will be a success. Moreover, it had been arranged that participants would attend as individuals, not as nominated delegates of national organizations.

But, Ucko went on to say, people do not appreciate that "you cannot now hold a conference and invite people from South Africa if you also hope that there will be people from up to thirty Third World countries". This is the reality, Ucko says that conference organizers cannot ignore.

The sequence of events leading to the

ban on South Africans, beginning towards the end of the 1984-85 academic year, is not easily reconstructed. Ucko cannot at this stage recall whether the first representations came from the students at Southampton (whose union and, in particular, debating hall, is required for the congress) or from the local branch of the Association of University Teachers. The formal decision of the Southampton City Council to withdraw financial support came relatively late, he said, although there had been informal representations earlier in the summer.

Ucko, like many others involved in the conference organization, says that people have not paid enough attention to the moral indignation of those opposed to South African participation. The University of Southampton has not considered the trouble caused by its proposed hospitality for next year's conference, and may not do so. The vice-chancellor, Mr G.R. Higginson, said earlier this week that the university had no formal status in the congress except as the provider for accommodation during the university vacation. But Mr Higginson added that he had told the organizers during the summer that he saw no alternative to their decision to ban South Africans once the opposition to their presence had become apparent.

Keyworth resigns as US science adviser

Washington

DR George A. Keyworth III, President Reagan's science adviser since 1981, announced last week that he is to resign from the position before the end of the year. Keyworth is widely credited with having successfully defended basic science even while other budgets were being cut, but also earned some unpopularity for

silence defences, which some fear could distort the pattern of financial support for basic research. But Keyworth said last week he was leaving at a time when everything was running smoothly: he was comfortable that SDI was on a firm footing and was encouraged by the general increased recognition of the importance of basic research, especially that conducted in universities.

William Carey, executive officer of the American Association for the Advancement of Science, said that Keyworth had earned a degree of access to the President that few other science advisers had achieved. He had also proven extremely effective in persuading the Office of Management and Budget to respect the importance of basic research, and had initiated important changes aimed at improving the efficiency of federal laboratories.

If Keyworth's term has been marked by generosity to basic science, his resignation comes at a time of widespread concern over the future level of support for science. Congress is considering legislation to reduce the federal budget deficit that would hit science hard, and many federal agencies have been told to expect budgets next year equal to or lower than this year's.

Keyworth is planning to establish a business with a former employee of the Central Intelligence Agency to advise companies on intelligence-gathering, where he is likely to command rather more than the \$70,000 salary he earned at the White House.

Tim Beardsley



being identified very much as a member of Keagan's team rather than as an emissary of the scientific community.

Keyworth has been a staunch supporter of President Reagan's controversial Strategic Defense Initiative (SDI) programme of research into anti-ballistic mis-

French research

Salomon speaks out (unpublished)

FRENCH technocratic centralism may have worked brilliantly for Ariane, nuclear power and high-speed trains, but it is not working, and will never work, for biotechnology, electronics and other consumer-oriented technologies. So argues Jean-Jacques Salomon, the only professor of science and technology policy in France, in a blistering — and unpublished — report now circulating at the Organisation for Economic Cooperation and Development (OECD), where Salomon was head of science for more than a decade.

Salomon's almost unrelieved criticism of French technology policy was commissioned 18 months ago by the prime minister, Laurent Fabius, in his then role of industry and research minister. Salomon submitted it to Fabius's successor, Hubert Curien, in July, but has heard nothing since. He is said to be furious that his passionate 160-page report may now simply gather dust.

The contents seem likely to guarantee that fate. For example, the report describes the creation of a grand ministry of research and technology by Jean-Pierre Chevènement as little more than "bureaucratic inflation". Scientists, writes Salomon, had much more influence over French policy in the pre-Chevènement days of the former and far smaller administration, the Délégation Générale de la Recherche Scientifique et Technique (DGRST).

The administrative consequences of Chevènement's expansion was a ministry split into several competing factions, says Salomon. This administration made only internal evaluations of its work, and rarely consulted its scientific advisers. Some advisory committees never met, according to the report, allowing bureaucrats to define programmes that were ends in themselves, not the means to innovation in the French economy.

In Salomon's report there are no scared cows, not even research spending. Basic research in France has enjoyed a 51 per cent budget rise since 1981 (without allowing for inflation), and applied programmes have seen a near doubling of spending. But according to Salomon, this attachment to a large increase in research and development spending as a fraction of gross national product has been "fetishism". (The ratio was 1.8 per cent in 1980, and 2.2 per cent now so a real increase has been achieved; the latest target is 3 per cent.) But, according to Salomon, the connection between research spending and economic growth is very loose: many other factors can matter more.

What the government should have been doing, according to the report, is actually to change the things that matter. But the author despairs whether in France anything can change. There have been 40 "re-

forms" of the French education system since 1945, Salomon counts, but none of them has finally changed anything. On the one hand, a true mass technical education remains out of reach despite frequent pious promises (of which the latest is Chevènement's, as education minister); and on the other, the top *grandes écoles*, the "engineering" schools that produce the elite of the French administration, in practice yield up graduates who "know nothing", neither science nor literature.

Also, Salomon suggests France must abandon its attachment to "great technological programmes" of state. These can only work when the state is, or controls, the purchaser; and can only fail when the purchaser is a multifarious public and the technologies various and rapidly changing (such as microelectronics). Here, the centralized French bureaucracy not only fails to be sensitive to the market but could not respond fast enough even if it were. The government should emphasize indirect action, where it has power and can change the climate, says Salomon.

• It is "indispensable" that there should be an independent, non-managerial, advisory unit to assess science and tech-

nology policy and ministerial action be established "as close as possible to the prime minister".

• The relevant ministries, with their competing and fragmented departments, should be streamlined, better directed to the marketplace.

• The "customer-contractor" principle, under which government departments should behave as customers for applied research by contract from research institutions, should be strictly applied to all big technology programmes.

• An external and public evaluation of the big technology programmes should be made "routine practice", as has become the case for the universities.

• The big programmes should take a lesser and lesser share of the cake, turning money towards "more supple and diversified" projects.

• Research and development aid to big industry must be analysed to determine if this is mere lame-duck support. Some 100 out of 1,500 French companies doing research receive 90 per cent of the government budget for industrial research and development, according to Salomon. There is danger of money going only to the strongest lobby, and more support is needed for innovative small companies.

• It is necessary to "rethink the French education system".

Robert Walgate

Cancer research

Ludwig branch lab to close

THE Ludwig Institute for Cancer Research is to close its London branch laboratories by the end of 1987. The unit, in collaboration with the Royal Marsden Hospital and the Institute of Cancer Research, is one of the major laboratories in the United Kingdom concerned with breast cancer. The Ludwig Institute has plans to open three more branches in London in the next two years and is negotiating with St Mary's Hospital, the Courtauld Institute and the Cardiothoracic Institute for sites. The staff at the present London laboratories in Sutton do not know whether they will be offered jobs at the new branches or even whether the organization intends to continue with breast cancer research. The reasons for the closure are obscure — clearly, lack of money cannot be one of them.

The Ludwig Institutes were set up in 1974 by Daniel Ludwig, one of the richest men in the world. There are at present nine branches, in the United Kingdom (2), Australia (2), Brazil, Belgium, Canada and Switzerland (2), and the organization plans to expand the number to 14. None of the branches are autonomous, but are administered by the Institute in Zurich. The present London laboratory was the first Ludwig branch to be established; its first director was Professor Munro Neville. Last year, Neville left to become a research administrator at the

organization's Zurich headquarters.

Staff at Sutton say that the post of director was not advertised after Neville left. The unit was without a director until the closure was announced, when Dr Tony Vickers, an ex-administrator from the British Medical Research Council, was appointed to oversee the rundown. Staff say that at the time of Neville's departure, the Ludwig executive assured them that the unit's future was not in doubt; eleven months later they received notice of closure. Although the technical staff have long-term contracts and are likely to be transferred, the scientific staff do not. And there is no indication that any of the new units will research breast cancer.

Scientific decisions by the Ludwig Institute are taken with the advice of a board of scientists from the United States and the United Kingdom. In the case of Sutton, staff are unconvinced that the normal procedure was followed and suspect that the decision was made at an executive level in Zurich. An explanation for the decision was promised for the staff by the end of November, but failed to materialize. Neville, speaking from Zurich this week, says that he "chose to leave" Sutton and that he knows nothing about the reasons for the decision to close it. And nobody else at the Zurich headquarters was prepared to explain the reasons for Sutton's demise.

Maxine Clarke

Embryo research

West German rules proposed

Bonn

THE long-awaited report of the West German interministerial commission on *in vitro* fertilization and related matters, set up in 1984 by the minister of research and technology, Heinz Riesenhuber, and the minister of justice, Hans A. Engelhard (FDU), was published last week (25 November). Part of the delicacy of the problem in West Germany arises because *in vitro* fertilization, even when carried out with the gametes of married couples, may be incompatible with the Basic Constitutional Law except when intended to lead to the birth of a child. The commission's proposals are to guide the drafting

of new legislation in the next few months.

The commission, chaired by the former president of the Constitutional Court, Ernst Benda, voted 17 to two for the majority opinion, which says that the use of donor sperm for *in vitro* fertilization may be permissible in special circumstances; the commission recommends that children conceived in this way should have a right to be told, at the age of 16, of the identity of the sperm donor.

In general, the report says, the technique should be used only for married couples who would otherwise be unable to conceive a child. The commission would strictly prohibit the use of *in vitro* fertilization for unmarried women, and the "rent a womb" practice in which fertile women bear a child for others for payment.

The most contentious of the recommendations are those concerned with the use of embryos in scientific experiments. In general terms, the commission would forbid embryogenesis for the purposes of scientific observation, but would allow state authorities the right to grant exceptions for investigations likely to benefit "the progress of medical science". The commission's findings have been criticized for not drawing a clear line. However the freezing of human embryos would be forbidden except in exceptional circumstances.

On the cloning of human embryos, the commission unanimously recommends a total ban, suggesting that such procedures should be made criminal offences. On gene therapy, the commission argues that attempts to alter the genetic constitution of somatic cells are in principle the same as organ transplants, and should therefore be allowed, but that gene transfer within the germ line should be prohibited.

One of the most controversial issues triggered by the appearance of the commission's report is that which would allow the analysis of the individual genome as when, for example, employers may seek to determine whether applicants for jobs are constitutionally unfit to do them. Although such checks are at present impracticable, the commission has been criticized by the trades unions and SPD for approving what may one day be possible.

One of the dissenting voices on the commission, that of Walter Doerfler, professor of genetics at the University of Cologne, argues that the commission's "basic attitude" towards new developments in human embryology is "very negative" and that emotions are a poor foundation for "hurried" legislation.

The other dissenting opinion is that of Professor Peter Petersen from Hannover, a psychologist. He is opposed to *in vitro* fertilization on the grounds that the physicians involved "do not really know what they are doing".

Jürgen Neffe

Danube dams

Austria takes responsibility

AUSTRIA is to take over responsibility for the construction of the controversial hydroelectric plant at Nagymaros, in Hungary, the Austrian finance minister, Dr Franz Vranitzky, announced last week. The cost, estimated at 8,000 million Austrian schillings (US\$450 million), will be borne by a consortium of Austrian commercial banks, and will be repaid, over 20 years, by electricity from the new power station. The deal, however, has been criticized by Austrian environmentalists, who feel that the contract unloads environmental problems on to Hungary.

The Nagymaros dam is the lowest stage in a joint Czechoslovak-Austrian peak-hour generating scheme. A storage reservoir at Dunakiliti, on the Danube below Bratislava, will, at peak hours, feed a generating station at Gabčíkovo, through a diversion channel that will take the main flow of the river away from the international border through Slovak territory. The Nagymaros dam, on the scenic Danube bend between Esztergom and Budapest, in addition to producing electricity for Hungary (and now, for Austria), will serve as a catchment for surges from Gabčíkovo.

Many Hungarian environmentalists have opposed the scheme, on the grounds that it will destroy the Danube wetlands which are the habitat of rare flora and fauna, ruin the amenity value of the bend (Budapest's chief resort area) and, by lowering the water-table, cause a pollution threat to the water supply of Budapest. The Hungarian government, the environmentalists urged, should either get Prague to agree to drop the scheme, or else withdraw unilaterally. Both courses, however, proved impractical politically, and in mid-August the Hungarian government reiterated its commitment to the scheme, though now with a postponed timetable and assurances of careful ecological monitoring.

Ironically, virtually the same environmental objections that the Hungarians had to the Dunakiliti-Gabčíkovo-Nagymaros installations were raised by the Czechoslovak government against Austrian plans to build a similar storage reservoir and power station at Hainburg.

In November 1984, the Czechoslovak foreign ministry announced that if the Hainburg dam were built, Czechoslovakia would demand massive compensation for ecological damage. A month later, after a long battle with the Konrad Lorenz Volksbegehren, Austria's main environmental organization, the Austrian government halted land-clearing operations at Hainburg, and, it now appears, began negotiating for a financial stake in Nagymaros.

Vera Rich

UK money for AIDS

MR Norman Fowler, Secretary of State for Social Services, this week announced that the British government would spend an extra £6.3 million on a package to combat the spread of acquired immune deficiency syndrome (AIDS). The money will be divided into five parts: £2.5 million for a national information campaign; £2.5 million for the Thames regional health authorities (75 per cent of the reported cases of AIDS in the United Kingdom are in London); £270,000 for six haemophilic counselling centres; £100,000 for training health professionals; and £750,000 for the Public Health Laboratory Service for testing blood samples for the AIDS virus.

The latest figures for the United Kingdom show that, of the 231 men and 10 women who have reported that they have the disease, 134 have died. A prediction of 1,837 new cases by 1988 was made at a meeting in London last week; the figure is higher than previous estimates because intravenous drug abusers are thought to be much more at risk than previously realized. The worry is that this group may be less amenable to public information campaigns than other "at risk" groups.

In a study published in the *Lancet* last month, it was reported that 38 per cent of serum samples from 106 Edinburgh drug abusers were positive for antibodies to the AIDS virus, with the proportions of men and women who registered seropositive about the same. Another report in the same issue analyses Centers of Disease Control data and shows that the relative risk of AIDS is four times greater for intravenous drug abusers than for homosexuals in the United States.

● The College of Health in London this week launched an addition to its Healthline service, which provides tapes of information on a range of medical subjects. Twelve new tapes give information on various aspects of AIDS; they can be heard by calling a London telephone number (1-980-4848) between 6 and 10 pm. Maxine Clarke

Polish universities

Government launches purge

At least 40 leading Polish university officials will lose their jobs as a result of the 1985 amendments to the Higher Education Act. The amendments increased the control of the Ministry of Science and Higher Education over the universities, and in particular, curtailed the rights of the universities freely to elect their own rectors, pro-rectors and deans. Despite assurances from government spokesmen last July that the changes then before the Sejm (Parliament) did not signal a purge in the universities, few academics were convinced. Their pessimism, it appears, was justified.

Under the amendments, rectors, pro-rectors and deans now in office will receive written confirmation of continued tenure from the ministry not later than the

end of January 1986, or else will be replaced. On 3 November, the underground newsletter of the pro-Solidarity Social Committee for Learning warned that the ministry planned to change the whole team of rector and four pro-rectors at Poznan's Adam Mickiewicz University.

This was surprising. Although the minister refused to confirm in office the rector elected in spring 1984, Professor Jerzy Fedorowicz, the current rector, Professor Franciszek Kaczmarek, a physicist, seemed an acceptable compromise.

Since then, however, there have been several pro-Solidarity incidents at the university. Moreover, the current Minister of Science and Higher Education, Dr Benon Miskiewicz, was the rector until his promotion to the post of minister shortly after the declaration of martial law. It seemed possible, therefore, that Minister Miskiewicz had singled out his own university as an example.

According to the Social Committee for Learning, the new rector was to be Professor Jacek Fisiak, a personal friend of the minister and a party hard-liner. Fisiak is a man of many parts. As head of the English department, he was responsible for the dismissal from the university, in 1978, of Dr Stanislaw Baranczak, the gifted poet and translator of English literature into Polish. He holds a British civil decoration, the Order of the British Empire, for his services to English studies in Poland.

Now, it appears that, according to Mr Andrzej Stolarski, chief spokesman for

the Ministry of Science and Higher Education, the new law specifically provides for a review of academic personnel throughout Poland, a statement somewhat at odds with the previous assurances. The Main Council for Higher Education, the supreme elected representative body of universities and higher colleges which, during the last academic year coordinated the campaign of the universities against the proposed revision of the law, met in closed session last Friday, but made no immediate statement. The Main Council, however, is known to be out of favour with the minister, who attacked it in parliament as being unrepresentative of the higher education community. It seems unlikely, therefore, that the Main Council will allow the dismissals to pass unquestioned.

Since the dismissals refer, so far, only to administrative posts, the rectors, pro-rectors and deans affected by the "review" will still, for the present, be able to carry on teaching — although there are strong rumours that the minister's next plan is for a "review" of teaching staff.

One rector who is to pay the price for putting his duty to his students before the demands of the authorities is Professor Wladyslaw Findeisen of Warsaw Polytechnic, who last year refused to identify and discipline those of his students who had carried the university banner at the funeral of the murdered Solidarity priest, Father Jerzy Popieluszko. The news of his dismissal last week evoked a massive and emotional demonstration by the polytechnic students, who gathered in the Great Hall of the Polytechnic. When the rector appeared, the students showered him with flowers.

Vera Rich

One US-Soviet pact

Washington

WHILE President Ronald Reagan and Mikhail Gorbachev were getting acquainted in Geneva the other week, another relatively unknown US-Soviet meeting was taking place in Moscow. Lee Thomas, administrator of the US Environmental Protection Agency (EPA), and Yuriy Izrael, chairman of the USSR State Committee for Hydrometeorology and Control of the Natural Environment, signed a memorandum presenting plans for cooperative work on 38 scientific and technical projects in the general field of monitoring the environment.

The meeting was the first high-level meeting on environmental cooperation between the two countries for six years. It took place under the auspices of a bilateral agreement originally signed in 1972 and since extended. Though the agreement has never been abandoned, deepening strains between the two countries had led to a steep decline in the number of scientists exchanged, from over 300 in 1977 to only 67 in 1984.

US officials say the United States has benefited in the past from the agreement, especially in earthquake prediction and environmental transport modelling. There have also been some difficulties: visas are often not available until the last minute, and last year one US official who had visited the Soviet Union several times under the agreement was suddenly denied a visa and slandered in *Izvestiya*.

At the recent Moscow meeting, eight areas of cooperation that had not been fruitful were abandoned, and four more were initiated. Nevertheless, the total number of scientific exchanges under the agreement is now expected to increase once more, according to Dr Gary Waxmonsky, EPA's executive secretary of US-Soviet programmes.

Tim Beardsley

Max Planck Gesellschaft

More cheer for Eureka than SDI

Bonn

THE Max Planck Society (MPG) seems eager that its 60 institutes and 10,000 employees should contribute to the European Eureka programme of technological research, but wary of the possibility that they might be caught up in the US Strategic Defense Initiative (SDI). So much emerged last week (27 November) when Professor Heinz A. Staab, the president of the society, introduced its annual report.

Professor Staab announced that the Max Planck Society is playing a leading part in one of the first Eureka projects, an inter-Europe collaboration in atmospheric chemistry. He said that the objective of the Eureka programme, at least as seen by MPG, is to complement the "existing one-way street to the United States" by more interconnections within Europe.

On SDI, Staab said that MPG has not so far been asked by the West German government to consider playing a part, so that MPG had not given formal consideration to the project. But he added that he could

not imagine that secret research could be carried out at any of the society's 60 institutes. He went on to say that plans for the development of basic research should not be "slipped over the scientific community" by politicians, whose influence should be used to strengthen existing collaborations.

MPG had funds of more than DM1,000 million to spend this year, and its budget of 1986 represents an increase of 3.8 per cent. But after allowing for the start-up costs of two new institutes, one for polymer research at Mainz and another for sociological research at Cologne, the increase for next year works out at only 2.8 per cent. Staab complained last week that the finance ministers of the *Länder* governments, which are responsible for half of MPG's budget, had originally worked for a mere 3 per cent increase of the total budget. Even now, Staab said, the increase would not be sufficient to cover the cost of inflation, reckoned to amount to between 5 and 6 per cent a year in the cost of research.

Jürgen Neffe

Planetary exploration

US Mars lobby gathers strength

Washington

THE "Mars underground", a network of enthusiasts dedicated to persuading the US government to support a manned mission to Mars, has again been raising its voice in public, pressing its case for a permanent base on Mars to the National Commission on Space. The signs are that it is being taken seriously.

The movement is made up both of university-based researchers and many in the National Aeronautics and Space Administration (NASA). As long ago as the early 1960s, NASA commissioned studies to investigate the feasibility of Mars missions, but enthusiasm waned as manned Moon landings became routine and the Apollo programme was brought to a premature close. But now the Mars underground seems to have found new strength.

Mr Leonard David, director of research of the National Commission on Space, is one of the biggest supporters of Mars ventures. He is to present a report on the future of the civilian space programme to the President and Congress by March 1986. The commission, appointed by President Reagan earlier this year, was explicitly told to take a bold long-term view, and it seems to be doing just that. It will look at options for the period to 2035 or even beyond, and will be taking a hard look at the Mars base plan as well as at proposals for a manned lunar base.

David points out that Mars has for many years been a vague long-term goal for NASA. With the shuttle now working at

least tolerably well and a space station apparently not too far off, the question that will arise is what they should be used for — whence the "case for Mars" group, which presented a 150-page summary of its plans to the commission last week at Stanford, California.

The group expects to use the first permanent manned bases on Mars established between 2010 and 2020; before then, though, bases may have been established on Mars's moons, Phobos and Deimos, which may contain enough water to use as a source of hydrogen and oxygen for fuel. The effort would be international and, after initial unmanned exploratory missions, the first crew of about 15 people would arrive for a ground spell of duty of up to two years.

The group lays much stress on using martian materials wherever possible, and notes that the martian atmosphere contains a high proportion of carbon dioxide, which could supply many essential needs for a permanent base.

The National Commission now has to weigh these ambitious plans against the rather less exciting lunar base plan. According to David, a lunar base might be a spin-off from a Mars base plan but is not generally thought to be an essential precursor. But there is one extra factor arguing for a Mars effort which could prove the most important of all: the Soviet Union has already spoken of plans for a manned Mars mission as early as the middle of next decade.

Tim Beardsley

Remote sensing

France puts trust in SPOT

SPOT 1, the first of two French observation satellites, is to be launched by Ariane on 11 January, only a few weeks late despite the failure of the last Ariane launch on 13 September. Arianespace, the commercializer of Ariane, will be crossing its fingers that adjustment to the design of a valve in the third stage will seal the leak of liquid hydrogen that caused the failure. So will the French space agency CNES, which has designed SPOT and which hopes it will steal the thunder from the US Landsat series of satellites.

CNES claims that twin high-resolution imaging instruments on SPOT 1, operating in the visible and near infrared, will be capable of identifying details down to about 10 metres, compared with the 30 metres of the last Landsat (Landsat 4) launched in July 1982).

CNES emphasizes, however, that the great innovation of SPOT is not so much its resolution as an ability to look away from the vertical. By this means, the satellite can in two passes some 90 minutes apart image the same Earth region from different angles, thus generating three-dimensional images. Simulations of such SPOT images have been made from aircraft, and they are certainly spectacular to view. These images should contain quite new cartographic information on vast regions of the more inaccessible parts of the Earth, and pose new challenges for information processing.

CNES has established a number of ground stations for receiving SPOT data, at which the data will be processed into directly usable images. In France, the principal station will be at Toulouse, where SPOT was designed and where all SPOT imagery is to be kept. A company called Spot Image has been established to sell and distribute images.

SPOT 1 will be accompanied into space, if all goes well, by the first Swedish scientific satellite, VIKING.

Subsequent Ariane launches are planned for February (two communications satellites, one for the US GTE Spacenet Corporation and one for the Brazilian government) and March (Intelsat V). Five additional launches are planned for 1986. Arianespace has also announced that despite its September setback, three institutions have since purchased future Ariane launches: one to orbit the first Luxembourg television satellite, which will broadcast to most of Europe, and one for a communications satellite for INMAR-SAT (the International Maritime Satellite Organisation).

These orders bring the total number of launch contracts of Arianespace to 37, of which 25 remain to be launched, the company says.

Robert Walgate

Parallel computers

GE aims at super-array contract

Schenectady, New York

GIANT General Electric (GE) is in the final stages of negotiating a contract with the US Department of Defense to complete the development and to begin the construction of what could be the world's most powerful computer.

The multimillion dollar project, a collaboration between GE's research and development centre here and the Artificial Intelligence Laboratory at the Massachusetts Institute of Technology (MIT), is for the construction of a machine with no fewer than 256,000 processors. Those responsible for the project wryly note that the new machine, called the Cross Omega Connection Machine, will have 100 times as much computing power as all the computers now installed at the centre.

The practical objective of the development is to construct a computer capable of processing in parallel the information in each picture element (pixel) of a standard 500 by 500 cell image. Suitably miniaturized, such a system could be carried by spacecraft or tanks and could make intelli-

gent decisions about the best response to incoming signals in video form.

The speed of the parallel array will be phenomenal — one million million operations a second. GE says that the architecture of the machine will be entirely novel, and that it will represent a milestone in the development of computing machinery. But the construction of the processors is also novel. By means of Very Large Integrated Circuit techniques, 64 processors will be incorporated on each of 4,000 semiconductor chips. By the use of optical lithography, GE claims that it now has in production at its plant at Research Triangle Park, North Carolina, integrated circuits with linewidths of only 1.2 micrometres, and that the research laboratory has made microcircuits with linewidths of 0.4 micrometres.

But Dr Phil Lewis, head of GE's computer science branch at Schenectady says: "We have to make a whole new computer science for this machine. And nobody knows how to do it."

Bill Johnstone

Japanese nuclear power

Straining at the US leash

Tokyo

JAPAN'S nuclear power industry, held back by US restrictions of fuel enrichment and reprocessing for more than a decade, has taken a great bound towards partial self-sufficiency in the production of its fuel, but probably in the wrong direction. Barely had the ink dried on an agreement with local officials to build a uranium enrichment plant based on centrifuge separation than the US Department of Energy sent shock waves through the Japanese industry by announcing that laser enrichment may be more economical. If this proves true, Japan's first commercial uranium enrichment plant due to open in 1991 will be outmoded and uneconomical.

Early this year, the governor of Aomori prefecture signed an agreement with Japan's Federation of Electric Power Companies for construction of a nuclear-fuel cycle complex in the prefecture on the northern tip of mainland Japan. It allows for plants for fuel enrichment and reprocessing as well as low-level waste storage. Although shaken by the US decision in June to switch to lasers, the Japanese government decided in October to press ahead with the enrichment plant next fiscal year, and a delegation was despatched to Washington in mid-November to seek revision of the 17-year-old Japan-US Atomic Power Agreement.

Behind these developments lies a 10-year struggle by Japan to break free of the US constraints which require "prior consent" before US fuel can be reprocessed. In 1972, Japan's Atomic Energy Commission recommended that commercial reprocessing plants should be developed after experience with a prototype plant, built by 1974 at Tokai-mura, north of Tokyo, by the Power Reactor and Nuclear Fuel Development Corporation (PNC), a semi-governmental research and development organization affiliated with the Science and Technology Agency.

With the tightening of US policy which the Indian nuclear explosion in May 1974, the start-up of operations at the Tokai plant was delayed by US restrictions until 1977, after Japan had signed the Treaty on Non-Proliferation of Nuclear Weapons. The delays by the Ford and Carter administrations raised bitter feelings, striking as they did at the heart of Japan's desires for energy self-sufficiency. PNC opened a centrifuge enrichment pilot plant at its Ningyo Toge works in 1979, and the steady trickle of fuel from this plant and the Tokai reprocessing plant has been used in PNC's prototype heavy-water reactor FUGEN and will be used in the prototype fast breeder reactor MONJU.

PNC has been less successful with its plans for radioactive waste storage. Last month it caused an uproar at Horonobe, a small town in the northern island of Hol-

laid, where it had planned a site for high-level storage with the approval of the town but in the face of opposition from the governor or Hokkaido, other local government leaders and residents. The Council of All-Hokkaido Labor Unions had mobilized a daily vigil by 300 members, reduced to 40 at weekends. PNC officials sneaked onto the site two weeks ago to choose sites for boreholes and for the location of a seismograph as well as to collect rock samples. A spokesman for the corporation said the inspection was carried out without notice to "avoid possible confusion among local residents".

Waste storage facilities will also be an integral part of the new complex to be built in Aomori. Around 66 million litres of low-level radioactive waste are at present stored at nuclear plants around the country. Earlier tentative plans to dump the waste in the Pacific from other Pacific countries, so that the Aomori complex will have storage space for up to one million 200-litre drums of waste in the first

stage and three million in the second have met with strong opposition. Proximity of the storage site on the Shimokita peninsula to the Misawa air base has raised fears of air-to-ground weapons going astray or crashing jets, but others favour the development as a stimulant to local economy.

Unlike the reprocessing and enrichment plants run by PNC, which are largely experimental in nature, the complex at Aomori will be a fully commercial enterprise backed by MITI and run by Japan Nuclear Fuel Industries Inc., a consortium established by the electric utilities and machinery companies in March this year. The plan is for Japan to produce domestically about 30 per cent of its enriched uranium needs by 2000. Japan would then be free to reprocess such "home-grown" fuel without prior consent from the US.

This panacea for Japan's dependence on energy imports may never materialize, however, if the laser-beam method of enrichment is successfully developed by the United States. Realizing this, the Japanese government announced in October plans to establish a joint government-private industry organization to develop the method.

David Swinbanks

Soviet *refusniks*

French scholarship in absentia

THE Paris-based Comité des Professions Médicales en faveur des Juifs d'URSS has established a bursary for *refusnik* scientists, taking the form of an invitation to study for a year in France. The award is to be called the Victor Hugo bursary to mark the appointment in 1882 of Victor Hugo as president of an emergency committee to help the Jews of the then Russian empire.

The first "recipient" will be Karen Khatchuryan, a 24-year-old ex-student of physics who was expelled from Moscow University in 1981 for "behaviour inconsistent with the name of a Soviet student" — in other words, for having filed an application to emigrate to Israel.

Whether the young man will be able to benefit from the award seems doubtful. If he was unable to obtain permission to emigrate to Israel, it seems unlikely that the Soviet authorities will let him go to France.

The bursary was announced during a meeting at the Paris Palais de Congrès devoted, in the first place, to medical problems relating to Soviet Jews. Some telling anecdotal evidence was presented on the decline in the proportion of Jewish school-leavers entering medical schools, particularly the distinguished establishments of Leningrad, Moscow, Kiev, Khar'kov and Vil'nyus.

Another panel dealt with the personal problems of the children of *refusniks*, forced to lead a double life between the Soviet culture they experience in school and the Jewish culture of their parents. Dr Albert Mallet, a psychiatrist who has vi-

sited the Soviet Union on several occasions, says that out of some 80 children examined, about half have developed serious personality disorders directly associated with their enforced lifestyle.

The third main panel at the meeting dealt with the political misuse of psychiatry. The case of Dr Zakhar Zunshain, a Jewish physicist from Latvia, suggests a change of Soviet practice. Undated copies of correspondence between Mrs Zunshain and the Deputy Minister of Health of the Latvian SSR, made public at the meeting, show that Dr Zunshain, a professor of physics who was sent to labour camp in March 1984 for having taken part in a demonstration outside the Bolshoi theatre in Moscow, had on some prior occasion been visited by a psychiatrist, at the request of the local police.

When Mrs Zunshain tried to obtain an explanation of this occurrence, the Deputy Minister, K. Berman, replied that it "should be considered quite normal". When denied a visa to emigrate, Dr Zunshain had refused to take "no" for an answer, and had continued to write "non-pertinent declarations which contained insults to the officials responsible", causing "serious doubts as to his mental health". The health law of the Latvian SSR, wrote Berman, provides for "prophylactic examination of citizens whose conduct and actions are characterized by a lack of critical spirit and opportunity", a ruling, which, if it were generally applied, could put the entire *refusnik* community at risk.

Vera Rich

Soviet seismology

Tajikistan earthquake reveals flaw

LAST month's earthquake in the Soviet republic of Tajikistan caused unnecessary damage, according to the local correspondent of *Izvestiya*, V. Surkov. The planning authorities of the Tajik SSR, he says, have for a long time "resisted" the classification of Leninabad *oblast'*, in the north of the republic, as liable to suffer earthquakes of force 8 on the Soviet 12-point scale. Not only had buildings been built to specifications suitable only for less seismic zones, he claims, but Leninabad city itself does not have a single seismic station.

Tajikistan is the most seismically active region of all the Soviet Union's 2 million km² of earthquake-prone terrain. The industrialization and development of the region has therefore called for considerable circumspection on the part of the geologists, and seismic risk maps for centres of population have been in existence for a number of years.



The latest forecast for Leninabad *oblast'*, which dates from 1982, increased the predicted maximum shocks from force 7 to force 8. The republic authorities, however, Surkov said, were reluctant to act on the new figures, and neither strengthened existing buildings nor modified the specifications for new ones. As a result, in the earthquake of 14 October, the towns of Kairakkum and Gafurov suffered heavy material damage.

Surkov's dispatch, from Leninabad *oblast'*, contradicts the impression given by earlier reports from the TASS correspondent, Aleksandr Nemirovskii, who filed from the Tajik capital of Dushanbe. According to Nemirovskii, "residential quake-proof houses built in recent years withstood the force-8 shock". This may be technically true — Surkov implies that some 10 per cent of housing was at least reparable — and Nemirovskii did go on to say that some people who had lived in old houses had had to be given shelter in tents, but the extent of the devastation, and the allegations of official negligence, come only from Surkov.

It is possible that even if the buildings had all been up to recommended standards, they might not have survived, since the shock in fact exceeded force 8. This means, Surkov notes, that future buildings in the area ought to be constructed to resist at least force 9. But some Soviet civil engineers seem doubtful about high-rise,

construction in seismic areas, and after the Gazli earthquake of March 1984 it was decided to replace devastated apartment blocks with one-storey dwellings.

More significant, perhaps, is Surkov's claim that Leninabad *oblast'*, which accounts for one third of the population of Tajikistan, has insufficient seismic monitoring stations. Earthquake prediction is a major concern of Soviet geology. Recommended methods range from sampling the helium content of geothermal waters to observing the behaviour of local fauna.

The most reliable early-warning sys-

tem, however, if populations are to be evacuated and emergency services alerted, is the continuous monitoring of microtremors, which are then processed by computer to establish forecasts of the expected epicentre and strength of imminent earthquakes.

In Leninabad *oblast'*, there were not only too few monitoring stations but the communications links to the Central Asian Seismic Forecasting Centre in Dushanbe did not operate. The earthquake itself, moreover, damaged telephone lines to the devastated areas, so that emergency rescue services, while fully aware that their help was urgently needed, had no idea where to send it.

Vera Rich

Japan in chaos

Radical attacks halt railways

Tokyo

As in other advanced nations, the functioning of Japan's giant cities is very much dependent on fast urban transport systems and the sophisticated electronic communications that control them. Just how vital these communications systems are was shown when left-wing radicals, protesting at plans to privatize the national railway network, last week cut trackside cables in Tokyo and Osaka. Eleven million commuters were stranded and for half a day many businesses ceased to function.

Almost exactly a year ago, a small fire knocked out computer links and brought Japan's banking system to a halt (see *Nature* 312, 393; 1984). At that time, there was an outcry over the vulnerability of the emerging back-up systems. But it seems that ultra-left groups learned more from that incident than did the government.

Early on Friday morning, radical groups, armed with garden shears and knowledge of where to find the cables for the national railways' central traffic control and automatic train stop systems, struck at 23 locations in the Tokyo area. The first the railway authorities knew was when screens at the central computer headquarters went blank; when they tried to contact key stations through their own telephone network, that too was found to be dead. Similar events took place in Osaka. That morning, the two cities' entire public rail systems were paralysed.

Repairing the cables is a difficult task: a minimum of four hours is required when a single break is made in the more sophisticated communication links. When there are several cuts in the same cable, the difficulties are multiplied many times over. During the day, huge traffic jams developed and enormous queues formed at the stations of the private railway line still running. One queue in Tokyo was said to consist of 35,000 people. Businesses opened late and a fifth of Tokyo's schools closed for the day.

In a related incident, other radicals,

dressed in business suits and ties but wearing helmets, attacked a medium-sized station in the centre of downtown Tokyo with Molotov cocktails. They then flung away their helmets and disappeared among the crowds of commuters. The station burned throughout the day.

No statement has been issued by the attackers, but it is assumed that they were members of the People's Revolutionary Army of the ultra-left Chukaku-ha (Middle Core Faction). Their attacks were timed to coincide with a strike by a break-away chapter of the National Railmen's Union based in Chiba, a little to the east of Tokyo, protesting against plans to privatize the National Railways.

The Chukaku-ha is a strong and well-organized revolutionary group with probably around 3,000 members, many of them recruited at universities. Membership of its secret revolutionary army probably numbers several hundreds.

Previously, the group has been most conspicuous in its bitter fight against the construction of Tokyo's new international airport at Narita. Although the airport was opened seven years ago, the radicals are still fighting for it to be closed and for land expropriated from small farmers to be returned. The airport is constantly guarded by some thousand police, but this has not prevented frequent disruptions in service.

That radicals now seem willing to use similar tactics against the railways is bad news for the Japanese government. Railway authorities have already admitted that there is no way they can guard hundreds of kilometres of communication cables against attack, nor can they install a complete back-up system without ruinous expenditure. Selling off the railways is going to be a considerably tougher task with radical opposition which could continue indefinitely. And police fear that the Chukaku-ha may be planning something big for the Tokyo summit of world leaders next May.

Alun Anderson

British civil plutonium

SIR—Barnham, Hart, Nelson and Stevens (*Nature* 317, 213; 1985) conclude that at least 2.3 ± 0.8 tonnes of plutonium produced by the magnox nuclear stations of the UK generating boards cannot be accounted for, and may have been diverted to weapons use. An earlier version of this paper was submitted by the Campaign for Nuclear Disarmament (CND) to the Sizewell B Public Inquiry where it was debated at length. The Central Electricity Generating Board (CEGB) demonstrated that the analysis was in substantial error and no credence could be placed on its conclusions or, of course, the implications that the authors were striving to secure therefrom. While the revised paper takes account of some of the comments made by CEGB, the main criticisms still apply. These are as follows.

(1) The paper derives the estimate of 2.3 tonnes by attempting to calculate the total plutonium from the CEGB and South of Scotland Electricity Board (SSEB) stations since 1963, and compares this estimate of 47 tonnes with the amount the government has accounted for in parliamentary statements, augmented by the amounts the authors estimate to be in civil use in the United States, and to have been lost in reprocessing. Since the approach depends on estimating the difference between two large numbers, the conclusion is critically dependent on the accuracy of the calculations — clearly the calculation of plutonium production needs to be accurate to better than 5 per cent for the claimed discrepancy to be of any significance. It follows that a very careful calculation is necessary to obtain results of the accuracy needed, but although the paper claims an accuracy of ± 2 per cent, this is not substantiated. In particular, an accurate estimate is needed of the plutonium production as a function of irradiation; this requires calculations for each station using detailed data on station design and operation, and a theoretical model more complex than the simplified method adopted in the paper, which uses approximate data culled from a variety of sources. Since the three different methods of calculation described in the paper all use the *same* estimate of plutonium production as a function of fuel irradiation (the function $G(B)$), the three methods are not independent. The degree of agreement between their results provides no evidence on the accuracy with which this plutonium production function has been calculated.

(2) The authors attempt to validate their overall method of calculation by comparing estimates of plutonium production over a 6-year period with data provided by CEGB for amounts of plutonium in irradiated fuel despatched for reprocessing from each of our eight magnox stations. Table 4 of the paper concludes that this shows that their model underestimates

plutonium production by 3.7 per cent, implying that their estimate of 2.3 ± 0.8 tonnes for plutonium unaccounted for is an underestimate by $0.037 \times 47 = 1.7$ tonnes. However, as CEGB explained at the Sizewell Inquiry, this comparison is not valid because it does not allow for changes in the level of irradiated fuel stocks at the stations over the six-year period. The comparison is also selective since the paper reports the comparison for seven of the stations but excludes the data provided for the other station at Wylfa. If Wylfa is included, the results are substantially changed — the overall ratio of plutonium dispatches to calculated production changes from 1.037 to 0.948. If the authors had not excluded the data for Wylfa, they would presumably have concluded that the comparison shows their method overestimates plutonium production by 5.2 per cent and that the claimed discrepancy of 2.3 ± 0.8 tonnes is *overestimated* by 2.4 tonnes.

The board is not able to publish a definitive analysis of plutonium production for reasons that were explained at the Sizewell Inquiry. These relate to the period before 1971 when some of the plutonium produced, although itself remaining in civil use, was bartered with the United States for a quantity of highly enriched uranium which was used for defence purposes. It has been the policy of governments over the years not to disclose this figure, and we are also precluded from providing detailed information which would help others to derive it.

To sum up, the accuracy of the authors' calculations is not sufficient to support the allegation that plutonium produced in civil reactors in the United Kingdom may have been diverted to weapons use.

Repeated statements in Parliament by ministers have confirmed that no plutonium produced in CEGB reactors has been used for military purposes in the United Kingdom or exported for use in weapons. The government has also stated that it has no plans to use plutonium produced in the board's reactors for nuclear weapons and this is also the policy of the US government.

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Free will

SIR—Nothing is gained by the invocation by D. H. Evans (*Nature* 307, 762; 1985) of the second law of hydrodynamics.

It may be true that free will implies the ability to think divergently; but determinism does not imply convergent thought: it implies only determinism. Given that the

thought processes of the scientist are the result only of causes in his environment, they can appear as convergent or divergent. They are convergent when the observer (who is also determined) perceives nothing new (in Evans' terms, no loss of entropy) which could not have been deduced logically from the observed facts. They are divergent when the observer perceives something new (either not deducible or apparently unrelated) which can subsequently be demonstrated by experiment. However, an observer with access to all the facts (that is, the whole internal and external environment of the scientist) might perceive the deductive logic of "divergent" thought.

Free will implies that the scientist has control over his divergent thought (which is intuitively absurd) while determinism implies only cause and effect. Convergence and divergence are then functions of the observer's environment, not the scientist's environment.

The entropy of the situation is more straightforward. Entropy is different in spatial and temporal regions of the Universe, and the variability might arise as a result of local fluctuations and aggregations. Given a spectrum of entropy, its value in one region is a statistical function (deterministically predictable but otherwise chosen (!)) and in any given region can be very high or very low. Thus in the spatial regions of living forms it can be very low: temporally it may continue to fall before it rises again.

Consciousness may indeed be a manifestation of biochemical processes, and the brain may function far from thermodynamic equilibrium: but that does not prevent it being determined in the same way that a whirlpool is determined.

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Academic tenure

SIR—The question whether those who work as professors or researchers at academic institutions should enjoy security of tenure has been much discussed (see, for example, *Nature* 317, 464; 1985) but surely, in democracies, if job security is to be eroded for some, the same rules should apply to others. Yet in the armed services, those who graduate from military academies and similar institutions have tenure for the rest of their lives. They may be asked to retire early but are compensated with a pension, and at no point are they required to justify their promotion, largely automatic, by enumerating the battles they have won or the missiles they have fired accurately, the military equivalents of papers published or graduate students trained. Can this be fair?

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Competition for big time opens

The launching of the Hubble Space Telescope next year will provide up to 3,000 hours of the best-ever telescope time. Here is how to bid for some of it.

THOSE hoping for a share of the time that will be available to users of the Hubble Space Telescope will at least now know to which address to write*. Towards the end of last month, the Space Science Institute on the Johns Hopkins campus at Baltimore put in the mail some thousands of copies of its formal *Call for proposals*, a document that explains the procedure applicants for telescope time must follow, together with more than a kilogram of supporting data, not to mention application blanks (twelve copies of which must be returned).

The documents give as full an account as there could be of how the potentially contentious issue of time allocation will be dealt with by the Space Science Institute. They are also, in passing, a vivid reminder of the potential power of the Hubble telescope, a 2.4-metre reflector equipped with instruments sensitive to radiation from the infrared (11,000 ångströms) to the ultraviolet (1,150 ångströms).

That the instrument will do more for observational astronomy than the building of the Hale telescope on Palomar Mountain in the 1930s seems generally accepted; it could yet outdo Galileo's contribution to the craft of observation. So competition for telescope time will be fierce.

So why will there be only 3,000 hours, rather less than 10 hours a day, of observing time each year? The new documents make plain the uncertainties that persist, the chief of which is that no date has yet been fixed for launching the telescope by means of the US space shuttle. And in the nature of things, nobody can guarantee that the instrument will find its way into orbit (at an altitude of 500 kilometres with an inclination of 28.5 degrees).

But the documents also explain that the efficiency with which the telescope can be reoriented towards novel targets is still an unknown quantity, whereas observations will not be feasible while the axis lies too close to the direction of the Sun, or the bright limbs of the outline of the Earth.

Applicants will also have to take account of the annoying complication of the South Atlantic Anomaly, the departure from regularity of the Earth's magnetic field that allows charged particles trapped in the magnetosphere to reach down

into the orbit of the telescope; the telescope detectors will be unusable not merely during passage through the anomaly but for an unquantifiable length of time thereafter, while the phosphorescence of the detectors makes the noise unacceptably high. One consequence is that the longest period of time when the continuous viewing of a target will be possible is estimated to be between 10 and 12 hours, a little more than at a high-latitude terrestrial observatory on a clear winter night.

The opportunities, on the other hand, are immense. The focal plane of the mirror is equipped with a variety of instruments, to each of which is assigned the radiation intersecting a predetermined patch of the plane, allowing the remainder of the radiation collected by the telescope to be gathered into the synoptic images of the wide-field camera (with the comparatively small field of view 154 arc-seconds square). This instrument will be able to acquire images, always in digital form, with a visual magnitude of 28. Perhaps more striking is the expectation that the use for astrometry of one of the three fine guidance sensors with which the telescope is equipped (two are needed for pointing the telescope) will allow angular positions to be determined to within 0.0016 arc seconds. This expectation, if borne out by experience, implies that it will be possible directly to determine the distances to important milestone stars used in constructing the distance scale of the Universe.

So how are these great opportunities to be shared? In principle, according to the Space Science Institute, anybody may apply for time. All proposals will be put through the same peer-review system, and time allocated on merit.

On the teasing question of deciding between the merits of proposals yielding quick spectacular results and long-term programmes bearing fruit only after the accumulation of a mass of data, the institute plans an arbitrary but workable solution. Each proposal will be considered by a disciplinary review panel, but final recommendations will be made by a Telescope Allocation Committee with general instructions to allocate roughly equal amounts of time to proposals in the categories small, medium and large. These allocations will account for roughly 90 per cent of the time, with the remainder reserved for allocation by the director, among other things so that unexpected opportunities may be seized and so that

the telescope may be used in novel ways.

The institute has already settled on three key projects to which special attention will be given, of which the chief is the determination of the distance scale, ideally so as to determine Hubble's constant to within ten per cent. The second consists of an invitation to exploit the potential of the wide-field camera so as to carry out a deep survey of selected regions of the sky, yielding information on such miscellaneous issues as the comet cloud beyond Neptune and the supernova rate in distant galaxies. Quasar absorption lines come third.

Speed will obviously be crucial. The documents make plain that applications for time will not succeed if the objective is a project identical with one already in the programme. Thus the first convincing proposal to measure Hubble's constant is likely to hold sway for two or three years. The other side of this coin of exclusivity is the institute's policy on the publication of data. At the outset, an observer will have exclusive access to the data gathered on his behalf for twelve months after the last of them have been stored, but thereafter, the data will become part of the general archive at Baltimore.

So how should intending applicants proceed? First, read the instructions carefully. The chances of being caught out by misunderstanding the technical performance of the instruments, or by miscalculating the length of the exposure required, are high. Those who succeed in the first round of applications will find themselves quickly asked for accurate positions of the guide stars they specify for their targets. Perhaps the best hope of winning time early is to go carefully through the list of projects put forward by those guaranteed exclusive use of the first six months (people who have helped to develop the telescope), looking for opportunities to make parallel observations of objects within the field of view of the Hubble telescope during that initial period.

But can it be worth the trouble when there is always a risk that the telescope may not be launched successfully? The risk of failure is no greater than that the shuttle itself might fail. But, whatever happens, the list of proposals on their way to Baltimore in the next three months is likely to serve future historians well as a record of the ambitions of the world's astronomers at this interesting time.

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Evolutionary biology

Sex ratios in wasps and aphids

from Robert M. May and Jon Seger

DARWIN seems to have framed most of the major problems in evolutionary ecology, and to have showed, at least in outline, how they might be solved. But he failed to crack the problem of the sex ratio. "In no case . . . would an inherited tendency to produce the sexes in equal numbers or to produce one sex in excess, be a direct advantage or disadvantage to certain individuals more than to others; . . . I formerly thought that when a tendency to produce the two sexes in equal numbers was advantageous to the species, it would follow from natural selection, but I now see that the whole problem is so intricate that it is safer to leave its solution for the future."¹ The future began in 1930 with R.A. Fisher; that it continues is shown by papers in *Science*² and this issue of *Nature*³.

Fisher pointed out that if we count grandchildren (rather than children), then it follows at once that there is an advan-

tage to producing the minority sex, whose members enjoy greater reproductive success, on average, than do members of the majority sex⁴. Thus males and females will be produced in equal numbers at the evolutionary equilibrium. If the cost of producing a male offspring differs from that of producing a female, then we need to state Fisher's result in its more general form, which says that at the equilibrium there is equal total investment in the two sexes. Darwin might easily have discovered this principle some 60 years before Fisher, had he thought to look ahead two generations instead of one when evaluating an individual's reproductive success.

Fisher's result assumes that there is population-wide competition for mates. In 1967, Hamilton⁵ showed that the ratio to investment may be dramatically female-biased under certain kinds of mating systems in which, owing to the physical

isolation of sibships, brothers compete to inseminate their sisters. Hamilton marshalled evidence demonstrating large female biases in a number of insects (mainly parasitoid wasps) that are likely to experience intense 'local mate competition' (LMC).

Hamilton's paper stimulated interest in many different kinds of problems concerning sex allocation. LMC itself has developed into a substantial industry, in part because its mathematics are seductively elegant, and in part because it explains patterns that do not have plausible alternative explanations based simply on the mechanics of sex determination. For example, it has often been claimed that the 1:1 sex ratios of mammals and birds are mere side-effects of the X-Y (or Z-W) chromosomal mechanism of sex determination. A recent book by Bull⁶ stands this reasoning on its head, by considering the evolution of a wide range of sex-determination mechanisms, taking into account their effects on the sex ratio.

Wasps have no sex chromosomes, being haplodiploid, so they can play

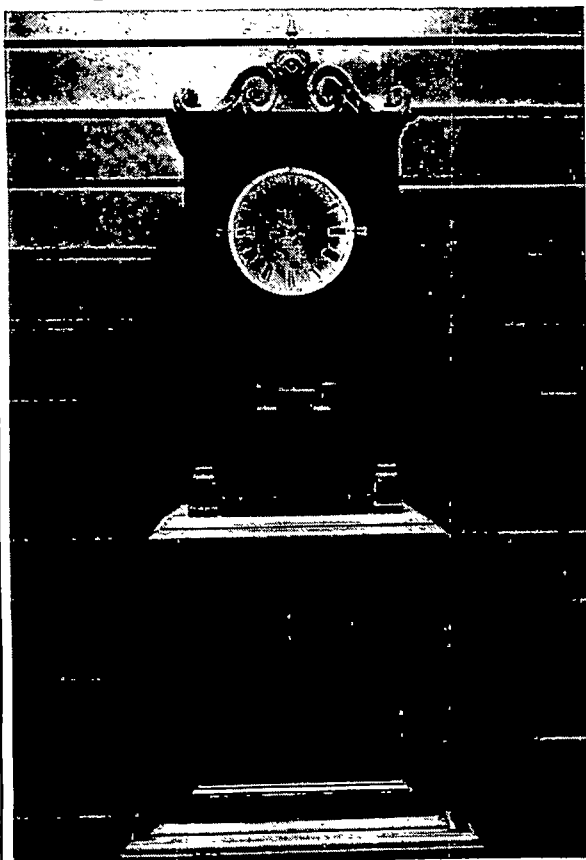
sophisticated sex-ratio games' involving extreme biases and, more interestingly, conditional responses to local variation in the environment or in the structure of the population. Theorists have explored the strategic possibilities at some length, and experimentalists have tested many of the resulting predictions using laboratory populations of the wasp *Nasonia vitripennis*, which has become the *Drosophila* of sex-ratio research. In no other field of evolutionary biology has it been possible to verify so many detailed quantitative predictions. Recent books by Charnov⁷ and by Trivers⁸ review the work on LMC and on many topics in the theory and practice of sex allocation.

Some of the most exciting recent advances have come not from the laboratory, but from the field. For several years there has been uncertainty as to whether the female-biased sex ratios seen under LMC are responses only to the competition for matings that occurs within groups of brothers, or whether they are also responses to the inbreeding with which such competition is almost always confounded. By comparing three species of fig wasps, Herre has recently shown that the biases reflect both the level of sib-competition and the average level of inbreeding⁹. Theoretical predictions, some derived for the first time by Herre, call for the more highly inbred species to show stronger female biases for any given number of foundresses in an individual fig (that is, for a given intensity of LMC). This is exactly what Herre sees in the three species. Not only are their sex ratios ranked in the expected order, but the actual data fall remarkably close to the theoretical curves relating sex ratio to foundress number. Herre's test of the subtle distinction between inbreeding and LMC was made possible by the existence both of variation within species in the number of foundresses per fig (supplying variation in LMC) and of variation between species in the average number of foundresses per fig (supplying variation in inbreeding levels).

A paper by Yamaguchi¹⁰ on page 460 of this issue also combines advanced theory and field data novel in at least two important respects. First, they add aphids to the list of major animal groups in which LMC appears to cause large sex-ratio biases. This is exciting news, in part because aphids have diploid genetics and chromosomal sex determination. How, then, do they control their sex ratios? The generation of aphids that infest plants throughout the summer are parthenogenic clones of XX females. But at the end of the summer, the last parthenogenic generation produces sexual females (who are XX) and males (who are XO). The male embryos are created by elimination of one X chromosome during a modified mitosis.

A second and strikingly novel pattern seen in Yamaguchi's data concerns sex-ratio differences among females who produce the sexual generation. These found-

Long-running experiments (II)



Although it has sometimes been stopped by mechanical failure, by the need for cleaning or when the ambient temperature has not fluctuated sufficiently, in principle this atmospheric clock has not needed winding since it was built in 1864. Now in the Physics Department of the University of Otago in Dunedin, New Zealand, its mechanism is driven by fluctuations in ambient temperature which expand and contract the air in an air-tight box. It is described in *European Journal of Physics* 5, 195-197; 1984, with a request for other examples of such experiments.

resses differ enormously in total fecundity yet, rather than producing the same proportion of males, each makes approximately the same absolute number. We are not convinced that Yamaguchi's simple and original theoretical explanation for this pattern captures all of the relevant features of the biology of the aphids, as it seems to assume that a female can assess the total fecundity of the foundress group to which she belongs; this would be reasonable for fig wasps, which produce their offspring after arriving at the fig, but may not be reasonable for the aphids studied by Yamaguchi, which produce their offspring before they fly to the trees on which the foundress groups are formed. But the pattern is there. It invites more theorizing and, of course, more empirical work.

XX-XO systems occur in many groups of arthropods and seem often to be associated with complex sex-allocation strategies (as in the laboratory nematode

Caenorhabditis elegans, which is mainly hermaphroditic, but with a small proportion of males). Yamaguchi's data should stimulate a widened search for instances of extreme sex-ratio manipulation among XX-XO species. Aphids themselves are economically important, so it may prove relatively easy to obtain support for studying their sex lives. □

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Quantum Hall effect

Dimensionality leaves its mark

from R.J. Haug

WHAT happens when one plays with the dimensionality of an object? This is not an esoteric question of interest only to mathematicians but a very important problem that has been actively explored by solid-state physicists in the past few years, not least because it is likely to lead to many totally new physical phenomena. Already, measurements of a two-dimensional electron gas (2DEG) in strong magnetic fields have revealed the quantum Hall effect (von Klitzing, K., Dorda, G. & Pepper, M. *Phys. Rev. Lett.* **45**, 494; 1980), for which von Klitzing was awarded this year's Nobel Prize in Physics. Now, H.Z. Zheng, K.K. Choi, D.C. Tsui and G. Weimann (*Phys. Rev. Lett.* **55**, 1144; 1985) have found a new, but related, effect — an anomalous variation in the magneto-transport coefficients of a two-dimensional electron gas as the width of the conducting channel becomes smaller than a characteristic length. This is of the order of 10 μm depending on the mobility of the electrons. The authors assume that the effect, which was only observed in high-quality samples, arises from a transition from two-dimensional to one-dimensional behaviour.

One of the reasons for this branch of research is the need for ever smaller components in the electronics industry. But, in addition, the modulation-doped GaAs/AlGaAs heterostructures used by Zheng *et al.* can also be used to fabricate very fast switches (Störmer, H.L., *Nature News and Views* **317**, 20; 1985). One obtains these structures by growing thin layers of GaAs, AlGaAs and AlGaAs doped with silicon on a crystal of GaAs by molecular beam epitaxy (MBE). Because of the

different electronic properties of the two semiconducting materials GaAs and AlGaAs, the electrons from the Si donors in the AlGaAs transfer to the GaAs layer, so forming a quasi-two-dimensional electron gas bound to the interface via the Coulomb attraction of the ionized donors across the junction.

An electron localized in a region of sufficiently small thickness behaves differently from a free electron that is able to move over large distances in all directions. If the electron is confined within a narrow potential well in the third direction, the quantized energy levels become discrete rather than being quasi-continuous. The electrons are unable to move freely in this direction. At low enough temperatures and sufficiently small carrier concentrations all electrons are in the lowest energy level; this is a description of a 2DEG. Owing to the spatial separation of electrons from their ionized parent impurities, the scattering rate is strongly reduced, which leads to exceedingly high mobilities, particularly in the low-temperature regime.

If a strong magnetic field is applied in the z direction perpendicular to a 2DEG kept at the temperature of liquid helium, and the voltages in the direction of the applied current (V_x) and perpendicular to this direction and to the magnetic field (V_y) are measured, a non-vanishing V_y is found in the magnetic field. This is the effect found in 1879 by E.H. Hall at Johns Hopkins University and it is true for all conducting materials. Von Klitzing observed that in a 2DEG at certain regions V_x vanishes, although there is still a current I through the sample and the Hall

voltage V_y shows a plateau. In such regions the relation of V_y to the current I is given by $V_y/I = h/\eta e^2$, where h is the Planck constant, e the charge of an electron and η an integral number.

Zheng *et al.*, like von Klitzing, measured V_x and V_y as a function of the magnetic field at low temperatures, with the aim of investigating size effects in the sample by changing the width of the conducting channel. First, they noticed that the quantum Hall effect is exact. When V_x vanished, the Hall voltage always showed a plateau with $V_y/I = h/\eta e^2$. This is another proof that the quantum Hall effect is a universal property of two-dimensionality of the charge carriers and does not depend on properties of the samples, such as their size.

But Zheng *et al.* also looked at the lineshape of their experimental curves in the region between the plateaus of the quantum Hall effect. When the electrons had low mobilities no difference was observed between wide and narrow samples. But for samples with very high electronic mobilities, the lineshape changed dramatically when the width of the channel was reduced, although the measurements with a wide conducting channel were still comparable to those obtained with low electronic mobility samples.

The data suggest that the new phenomenon arises as the size of the sample approaches some characteristic length of the electronic system. One possible explanation put forward by Zheng *et al.* is that the characteristic length is that of the inhomogeneities in the sample. Another possibility is that it is associated with the electron-electron interaction, in which case the observed effect would have to be attributed to a dimensional crossover from two-dimensional to one-dimensional behaviour. In other words, if the channel is narrow enough the electrons cannot move freely in two dimensions: one direction is open but in the other motion is restricted by the edges of the sample.

These explanations are, however, not definitive. In experiments with silicon metal-oxide semiconductor field-effect transistors (Si-MOSFET), where samples of different channel length but identical channel width have been measured (von Klitzing, K. *et al. Proc. 17th Int. Conf. Physics Semiconductors*, San Francisco, 1984), very long samples produce results that are strikingly similar to those of Zheng *et al.* for very narrow GaAs/AlGaAs samples, whereas short samples do not. For a long sample it is difficult to explain the effect by a dimensional crossover as motion is not restricted in one direction. Nevertheless, the experiments give hints about the underlying fundamental physics. Further experiments and ideas will clarify these problems. □

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Cell biology

Organizing the nucleolus

from John Sommerville

THE function of the nucleolus is to produce ribosomes in response to the physiological demands of the cell. Nucleoli develop from chromosomal sites, known as nucleolar organizing regions (NORs), which contain tandemly arranged genes for ribosomal RNA (rRNA). The initiation and extent of their development is subject to both cell-cycle and metabolic influences. At a recent meeting* substantial advances were reported in identifying the components involved in nucleolar activity and in describing the interactions that occur between them.

After mitosis in a cell actively synthesizing proteins, the nucleolus is generated from the NORs as a massive and complex structure, bringing together all of the components required for the manufacture of ribosomes (see the figure). As well as transcription of the rRNA genes by RNA polymerase I under the control of associated transcription factors, this process involves many other components produced in other parts of the cell and brought into the nucleolus. These include about 80 ribosomal proteins, small RNA species required for rRNA processing (U3 and U8 small nuclear RNAs) and ribosome maturation (5S RNA), and several acidic proteins required for the maintenance of nucleolar structure and the assembly and transport of preribosomes. As these components have to be manufactured at a rate determined by the demands of ribosome production, considerable co-regulation of gene transcription is required.

Ultrastructural analysis combined with autoradiography to detect the initial sites of RNA synthesis (G. Goessens, University of Liège) and with selective silver staining to detect DNA- and RNA-associated proteins (K. Smetana, Czechoslovak Academy of Sciences, Prague) has made it possible to map the stages of ribosome formation to distinct regions in the nucleolus. The processes of initiation, production and maturation seem to progress from centre to periphery and the consensus is that the rRNA genes of the NORs are located in regions of low electron density (fibrillar centres), that gene transcription is initiated here but progresses into the surrounding dense fibrillar component where proteins bind to the transcripts, and that processing and maturation of pre-ribosomal particles occur in the cortical granular component.

A key event in initiating nucleolar development at the NOR is the activation of rRNA genes. It has been shown from immunostaining studies (U. Scheer, DKFZ Heidelberg) that substantial amounts of RNA polymerase I are located at NORs during mitosis, permitting rapid activation

of the genes as cells enter interphase. From *in vitro* reconstruction experiments using cloned genes and cellular extracts, the sequence of events in initiating the transcription of mammalian rRNA genes has been established (M. Muramatsu, University of Tokyo). First, a species-specific DNA-binding protein — transcription factor ID (TFID) — binds to the gene-promoter region located just before the transcription start site. A second, growth-dependent transcription factor (TFIA) then acts through protein-protein interactions to bring RNA polymerase I to the promoter. After this initiation complex is formed, both TFID and TFIA remain bound to the promoter region while RNA polymerase I reinitiates many rounds of transcription. It is interesting to note that point mutations at 7, 16 and 25 nucleotides before the transcription start site reduce activity to 0, 10 and 50 per cent of wild type, respectively. This nine-nucleotide interval could represent a linear series of protein contact points along the DNA double helix.

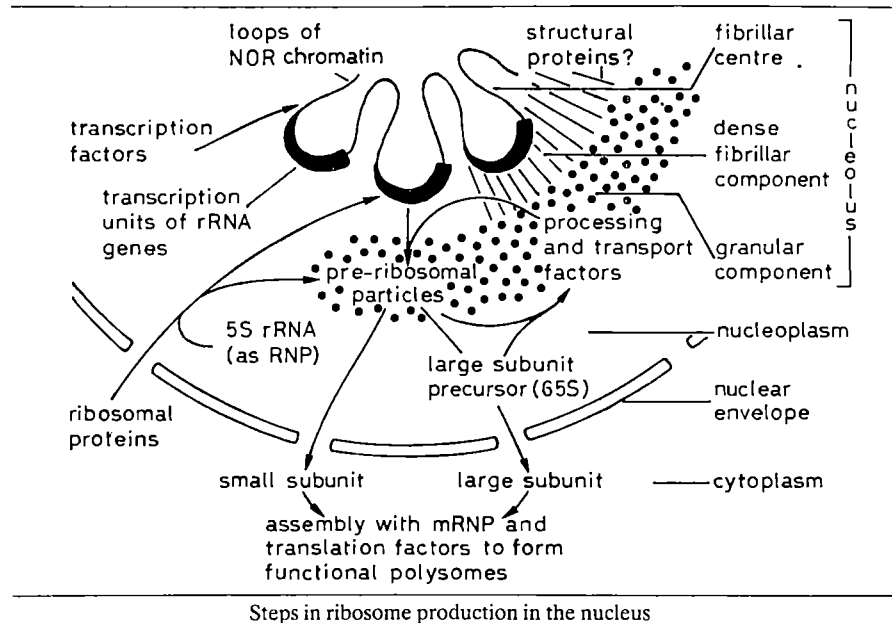
Attraction of transcription factors to promoters in *Xenopus* seems to take a special route (R. Reeder, Hutchinson Cancer Research Center, Seattle). In the spacer regions between rRNA genes, there are multiple 60 or 80 base-pair (bp) enhancer elements, a block of which confers a 20-fold increase in transcription activity compared with genes lacking enhancers. In gene constructs, these elements are effective when they are located 4–5 kbp from the gene promoter; they can be in either orientation and can be located in the gene itself. They seem to function as sites for the attraction and

entry of a transcription factor that is transferred to the gene promoter to form the transcription initiation complex.

Transcription of rRNA genes in *Xenopus* terminates 235 nucleotides beyond the end of the gene (Reeder). Using short DNA probes from this region, a stable RNA species corresponding to this extra transcription is detected, which tallies with electron microscope observations (M. Trendelenburg, DKFZ Heidelberg) of three additional polymerase molecules at the end of the rRNA transcription unit, with no long transcripts attached. It would appear that the rRNA transcripts are rapidly processed by cleavage before transcription terminates. Discovery that RNA polymerase I uses enhancer sequences and 3'-processing of transcripts brings its activities more into line with those of RNA polymerase II.

Of the many proteins that associate with rRNA gene transcripts at various stages of their processing, the best characterized are the ribosomal proteins (rps). Features contributing to the co-regulation of nucleolar rRNA genes and the many *rp* genes scattered throughout the genome have been studied in yeast (W. Mager, Vrije University, Amsterdam), *Xenopus* (E. Beccari, University of Rome) and mouse (K. Dudov, Bulgarian Academy of Sciences, Sofia). The *rp* genes of these organisms are similar in that they often lack a conventional TATA promoter sequence, having instead a long pyrimidine tract running through the transcription start site. In most of 22 *rp* genes in yeast that have been sequenced completely, there are two 12-bp-long homologous sequences implicated in transcription activation, located 300–500 nucleotides upstream of the initiation site.

Co-regulation of the primary rRNA-binding *rp*, L25 has been studied in detail by Mager. By increasing the dosage of L25 genes in yeast cells using a shuttle vector, it is found that L25 transcription is not



co-regulated with endogeneous *rp* genes; instead excess L25 is synthesized, which in turn may block further translation of its own message. A link between nucleolar activity and L25 gene expression is provided by a homologous sequence present in 26S rRNA and L25 messenger RNA, either of which can bind the L25 protein.

The yeast system has similarities with the regulation of expression of *rpL1* in *Xenopus* (Beccari). Accumulation of L1 resulting from an increase in *L1* gene dosage by microinjection into oocytes, leads to blocking of the removal of introns from the *L1* gene transcript. A regulatory sequence is again implicated, here located in the introns of the *L1* gene transcript and in 28S rRNA. Binding of excess L1 to its own gene transcript would effect autogenous regulation by blocking processing and hence further production of L1 until it is removed by binding to rRNA.

In the nucleolus, the predominant proteins are neither transcription factors nor individual rps. Of particular interest are the silver-staining phosphoproteins with relative molecular masses around 100K. Their amino-acid sequences, derived from protein chemistry (H. Busch, Baylor College, Houston) and cDNA sequencing (M. Caizergues-Ferrer, CNRS, Toulouse), contain conserved functional domains. One, rich in glutamic and aspartic acids, is thought to interact with chromatin; another, rich in glycine and dimethylarginine, is found in various RNA-binding proteins. Because they are only transiently associated with rRNA gene transcripts and are lo-

cated in the inner fibrillar regions of the nucleolus, these proteins seem to be involved in relatively early stages of rRNA processing.

A completely different acidic nucleolar protein of 40K (B. Hügler, DKFZ, Heidelberg), associated with preribosomal particles in the cortical granular component of nucleoli and with the nucleoplasmic 65S precursor of the ribosomal large subunit, seems to be functional later. As it is never found outside the nucleus, its primary function seems to be the transport of preribosomal particles. Another important difference is that the silver-staining proteins, together with RNA polymerase I, remain associated with the NOR during mitosis whereas the 40K protein is distributed with some ribosomal protein over the entire surface of the chromosomes. Other nucleolar proteins are distributed into the cytoplasm during mitosis.

Obviously the mechanisms involved in bringing these dispersed components together to construct new nucleoli require much further study. One way into the problem might be to create novel NORs by microinjecting cells with recombinant plasmids containing rRNA genes that would fuse and integrate into chromosomal sites. This approach might also tell us whether intranuclear positioning is important — does the NOR have to be brought into close association with the nuclear envelope before nucleolar development can proceed? □

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100 years ago

The fresh-water mussel closes its shell by contraction of two strong muscles — one before and one behind — but how does it open its shell? This has recently been studied by Herr Pawlow. The animal was fixed on a board by one shell, while the other shell was connected by a silk thread with the short arm of a lever — the longer arm of which indicated the movements on a slowly-rotating drum. . . there are two classes of nerve-fibres connected with the muscles — the one motor, producing contractions; the other inhibitory, producing relaxation

From *Nature* 33 106, 3 December 1885

an event that took place in the late Pliocene, 2.3 Myr ago. A search in contemporaneous sediments recovered from the North Pacific sea floor has failed to detect an equivalent siderophile anomaly. Although the exact spatial distribution of the Antarctic debris is unknown, the event was clearly not global in scale, unlike the event thought to have occurred at the Cretaceous–Tertiary boundary.

The estimated infall of meteoritic material in one of the Antarctic cores is about 100 mg cm^{-2} . This rivals the most recent estimate of the average global outfall of meteoritic (chondritic) material of about 140 mg cm^{-2} at the Cretaceous–Tertiary boundary by F.T. Kyte, J. Smit and J.T. Wasson (*Earth planet. Sci. Lett.* 73, 183; 1985). In this core, enrichments in the siderophile element iridium are obviously correlated with the meteoritic material and reach a maximum of approximately 5 ng g^{-1} . This is roughly an order of magnitude less than the maximum values reported for the Cretaceous–Tertiary boundary at Caravaca, Spain. Variation in the relative abundances of siderophiles is a geochemical characteristic of various meteorites and the difference indicates that the Antarctic projectile differed in composition from that postulated to have caused the Cretaceous–Tertiary iridium anomaly.

Because the Antarctic debris is of relatively coarse-grained nature, with particles up to several millimetres in diameter, the type of projectile can be identified with some confidence. Three types of particle have been recovered: vesicular glasses; brecciated and shocked basalt; and Fe–Ni metal. Most of the iridium is in the vesicular particles, which make up more than 90 per cent of the recovered particles and contain about 150–200 ng iridium per gram. The vesicular particles have some textural similarities to meteor ablation spheres but their composition is similar to that of basalt. The identifiable basalt particles consist primarily of the minerals plagioclase and pyroxene and are often enclosed in vesicular material. The basaltic particles could originate either from projectile material or from ocean-floor basalts. Although no chemical analyses are presented, it is clear from the mineral chemistry, particularly the manganese content of the pyroxenes, that they represent material from the group of meteorites termed basaltic achondrites, probably of the howardite type

Meteorites

Siderophile-enriched sediments and meteoritic debris

from Richard A. F. Grieve

Low sedimentation rates make deep-sea sediments a relatively rich depository for the extraterrestrial materials that bombard the Earth. F. T. Kyte and D. E. Brownlee (*Geochim. cosmochim. Acta* 49, 1095; 1985) give details of an important find of meteoritic debris in the Antarctic Basin, which provides evidence that sediments chemically enriched in siderophile elements, those elements that tend to concentrate with iron, are associated with meteoritic material. This discovery, initially reported in *Nature* (292, 417; 1981), clearly demonstrates that large impact events can produce siderophile-enriched sediments, the postulated explanation for the origin of the iridium anomaly found at the Cretaceous–Tertiary boundary.

Siderophile elements, which include

cobalt, nickel, iridium and platinum, occur preferentially with native iron. As a result, they are relatively depleted in the Earth's crust and concentrated in the core. Certain meteorites, however, are relatively rich in siderophiles, and local enrichments in siderophiles have been found in so-called impact melt rocks associated with some terrestrial meteorite impact structures. It had been suggested that siderophile-enriched sediments could also result from the presence of meteoritic material but until now no direct association of siderophile-enriched sediments and meteoritic material had been observed.

The meteoritic material was discovered in two piston cores, recovered from water approximately 5 km deep at sites 120 km apart, at 57°00'S, 89°12'W and 57°47'S, 90°48'W. Palaeomagnetic stratigraphy indicates that the meteoritic debris is from

*The Ninth Nucleolar Workshop, Kraków, Poland, 13–17 September 1985.

This poses a problem. Howardites are differentiated meteorites; that is, their source body underwent large-scale chemical differentiation with the formation of an iron-rich core and a silicate crust. Thus the howardites, which represent the brecciated silicate crust, are relatively depleted in siderophile elements. The basaltic clasts, therefore, cannot be the source of the siderophiles in the vesicular material. The most obvious source is the rare-metal grains. Calculations using a mixing model indicate that the vesicular material could correspond approximately to howarditic material with a 3 per cent metal component. The metal composition required by the mixing model does not correspond exactly to that determined for the cores. This is not, however, a major difficulty, as only one grain was analysed and changes in metal composition caused by oxidation reactions during impact have been documented at terrestrial impact sites.

If the vesicular glasses are a mixture of howarditic material and metal, with a minor addition of seawater, what was the thermal event that led to melting? Heating during atmospheric entry or impact with the ocean are two possibilities. Although they present no compelling arguments, the authors favour the latter. Impact as the heat source is reasonable, at least from the point of view of physics. If impact occurred at 15 km s^{-1} , peak shock pressures would be of the order of 150–200 Gpa, with the attendant post-shock temperature well above the melting temperature of howarditic materials. If the thermal energy

associated with impact was sufficient to produce the vesicular particles, then why are they intimately associated with unmelted basaltic particles? The authors suggest the projectile suffered at least partial break-up in the atmosphere. Smaller pieces would be decelerated by atmospheric drag, so that their impact velocity would be below that required for melting, and would recombine with the melted vesicular material through collisions in the cloud of ejecta. The size of the projectile is poorly constrained at approximately 100–500 m in diameter, on the basis of the distribution of meteoritic material. A projectile in this size range would be subjected to some atmospheric crushing and dispersion, according to the calculations of H.J. Melosh in *Multi-Ring Basins*, 29 (Pergamon, 1981).

From recent calculations of the terrestrial cratering rate, it is estimated that as many as 50 similar events occur in the Earth's oceans every million years. As ocean-floor sampling continues and deep-sea sediments are examined, it is likely that other oceanic impact events will be discovered. This Antarctic discovery not only strengthens the meteoritic origin of the siderophile enrichments at the Cretaceous–Tertiary boundary but, together with potential future discoveries should also provide insights into the complementary subjects of meteoritics and impact phenomena. □

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Ecology

Nuclear DNA content as a guide to plant growth rate

from Peter D. Moore

ONE of the most clearly defined aspects of the ecological niche of a plant is the temporal element in its seasonal growth and development¹. Even if all the plants in a meadow are tapping the same energy resource, they can partition that resource in time by expanding their leaf canopies in sequence. A knowledge of such differences can be of considerable importance in the management and conservation of grasslands where decisions must be made concerning the timing of mowing or grazing regimes². As a result of the recent work of Grime, Shacklock and Band³, it is becoming apparent that the timing of the growth of the shoot systems of many grassland plants is closely related to their nuclear DNA content, which means that anatomical examination can provide considerable information about the nature of niche partitioning within a plant community.

Grime and Mowforth⁴ had already suggested that the nuclear DNA content of certain plants is related to the timing of

shoot growth. Having observed that Mediterranean species of plants, which have their main growth periods in winter and early spring, have a high nuclear DNA content, they examined a wide range of British plant species to see whether any relationship between DNA content and time of growth was apparent. The found that species like *Hyacinthoides non-scriptus* (bluebell) and *Ranunculus ficaria* (lesser celandine) that commence growth in the early spring (April) have DNA contents that are about ten times as high as those that commence growth rather late in the season (June), like *Urtica dioica* (stinging nettle) and *Molinia caerulea* (purple moor grass). The capacity to grow at lower temperatures is thus associated with the possession of a large genome.

But the evaluation of the full ecological implications of this proposal required the analysis of an area of vegetation under natural field conditions, which is what Grime and his co-workers have now com-

pleted. They chose to examine the nuclear DNA content of plants growing in a north-facing area of damp limestone grassland in North Derbyshire, England, that was particularly rich in herbaceous plant species. They removed 150 circular turf samples of 10 cm diameter and maintained them under garden conditions, where they were able to establish the rates and times of leaf elongation for all the major species. At the same time they monitored temperature, and measured nuclear DNA content in samples of plants' root tips.

The nuclear DNA contents of the plants was fairly evenly distributed over a wide range from less than 1 pg to almost 40 pg per nucleus. In the cold conditions of early spring (March), species with more than 10 pg DNA per nucleus grew two to four times as fast as those with a less than 10 pg per nucleus. By the beginning of May, the growth rates of the two groups were only slightly different and by June the differences were entirely insignificant.

The experiment clearly demonstrates the limiting effect of temperature on leaf extension in the early part of the year and also the advantage gained in this period by species with a high nuclear DNA content. The authors regard a small nuclear DNA content as characteristic of plants which grow by rapid and continuous cell division during favourable periods (such as the summer season in temperate areas). Rapid spring growth is best achieved by expanding cells which were produced during the previous growing season but which have remained unexpanded during the intervening winter. The problem of conducting mitosis under cold conditions is thus avoided. Such plants characteristically have high nuclear DNA contents. Their growth declines in summer when they conduct repeated cell divisions to produce tissues which will expand during the following spring.

From an ecological point of view, one of the most interesting points to emerge from this work is the chance it offers to catalogue at least one dimension of niche specialization simply on the basis of cytological investigation of the plants in a community. An examination of the spread of DNA values in a community, for example, provides an insight into the extent that the various species are exploiting the range of temporal opportunities available to them.

Now that it has been demonstrated that the broad relationship between DNA content and the time of canopy expansion actually operates in a natural community, it should be possible to make informed predictions about the temporal role of species in various communities on the basis of their DNA content. As Grime and his colleagues point out, DNA analyses may also provide a clue to the vexed question of how certain vigorous community dominants can coexist. For example, the fact that one robust grass, *Brachypodium pinnatum*, has a nuclear DNA content of

2 pg, whereas another, *Bromus erectus*, contains 22 pg per nucleus, may explain the way in which these species partition the environmental resources and thereby manage to coexist. Studies of this kind will assist botanists to appreciate more fully the implications of Gause's competitive exclusion hypothesis in the realm of vegetation. □

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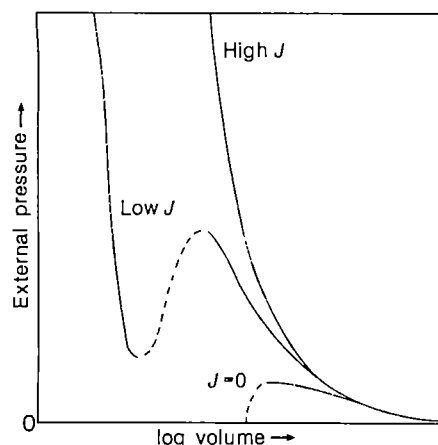
Astrophysics

Phase transitions in star formation

from Alan P. Boss

MANY dynamical aspects of star formation have become understood through theoretical models of collapsing interstellar clouds but attention has recently been turned to equilibrium models. From models of the possible equilibrium states available to interstellar clouds and their protostellar progeny, it emerges that two types of equilibrium may occur for isothermal clouds with small angular momentum, with one being much denser than the other. Therefore, if there are phase transitions — mathematically analogous to the transition of a Van der Waals gas to a liquid — from the more diffuse to the more compact state, the result could be star formation in clouds that would otherwise be considered stable¹.

An interstellar cloud must contract and increase its density by a factor of about 10^{23} in order to form a star. Observation has yielded little knowledge about the contraction process, because of the inherent obscuration and small length scales involved. Theoretical models of nonmagnetic star formation have focused on solving the exceedingly difficult time-dependent problem of the collapse of an interstellar cloud from an initially unstable state.



Axisymmetrical equilibrium sequences for isothermal clouds with differing amounts of angular momentum, J . No equilibrium exists for sufficiently dense clouds with $J = 0$. Clouds with low J can exist in either a diffuse or compact state, separated by unstable equilibria (broken line). For high J clouds the unstable branch disappears. Phase transitions from the diffuse to the compact branch may initiate star formation (modified from ref. 2).

To include rotation and the possibility of fragmentation, the model must include all three spatial dimensions, a formidable task. Protostellar clouds are effectively isothermal during much of their evolution, but eventually they become opaque and heat up, adding further to the complexity of the computations.

A simpler approach is to seek equilibrium states for rotating, isothermal, axisymmetrical, self-gravitating clouds (see figure). Models based on the global properties of spheroids of uniform density reveal that, for clouds with low angular momentum, two types of equilibrium are possible²⁻⁴: slowly-rotating, nearly-spherical clouds, supported primarily by external pressure (diffuse branch), and rapidly rotating, highly flattened clouds, supported largely by rotation (compact branch). Intermediate configurations are unstable to compression. For clouds with large angular momentum, a single sequence exists, extending monotonically to high densities.

The diffuse state is metastable, because its free energy is higher than the compact branch. The unstable branch has the highest free energy, forming a barrier between the stable branches. If the free energy of a diffuse branch cloud is raised sufficiently, such as by compression in the shock front associated with the spiral arms of our Galaxy, an otherwise stable cloud could dynamically become a compact cloud. Such a phase transition would allow low angular momentum clouds that are stable against collapse to become significantly denser¹.

Phase transitions can only be considered physically possible if detailed models of the two branches can be constructed and shown to be dynamically stable. Rigorous modelling of clouds with external pressure produces stable clouds that become progressively more condensed at their centres as the mass increases⁵. The models become unstable to compression at high central density, as in non-rotating, isothermal clouds supported by external pressure. These models are the realistic manifestation of the diffuse branch³.

Analytical solutions exist⁶. They are compressionally unstable when the cloud is nearly spherical, and dynamically un-

stable to ring formation when highly flattened⁷; intermediate models are stable to axisymmetrical perturbations. However, these solutions are mathematically singular at the origin and extend to infinity. Because of the lack of external pressure, the flattened solutions are similar to the compact branch. Thin-disk models have been constructed which also suffer from an infinite central density, but they do include external pressure. These models are the closest yet developed to a rigorous description of the compact branch. Because compact branch models with finite central density have not yet been constructed, and their stability to axisymmetrical perturbations has not been tested, it is premature to declare the compact branch a possible member of the physical universe. It is important to note that calculations of the dynamical collapse of isothermal clouds have found the diffuse branch⁸ but not the compact branch. Instead, collapsing axisymmetrical clouds produce either rings or runaway disks, depending on details such as the initial distribution of angular momentum.

This discussion has dealt with stability to compressional or axisymmetrical perturbations but interstellar clouds can also distort in a non-axisymmetrical way. Simplified ellipsoidal models form a high angular momentum sequence (similar to the diffuse branch) that terminates when sufficiently compressed. The simplified compact branch models are usually dynamically unstable to non-axisymmetrical distortions which could result in fragmentation and further contraction to the stellar state⁹. The stability of detailed models of the compact state to non-axisymmetrical distortion is unknown.

If phase transitions occur between diffuse and compact interstellar clouds, it may be easier than has been supposed for the process of star formation to be initiated. Clouds that are stable to small perturbations may be hammered into a dynamic collapse towards a denser configuration. A collapsing cloud may well bypass any compact equilibrium state and fragment directly into a protostellar system. The validity of these speculations awaits confirmation of the existence and stability of the compact branch. Bear in mind, however, that unstable equilibrium states are of considerably less interest to nature than to theoretical astrophysicists. □

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New strategies for AIDS therapy and prophylaxis

SIR—In search for a reactive agent against HTLV-III/LAV, the virus that causes AIDS (acquired immune deficiency syndrome), the attention is focused on neutralizing antibodies. Such antibodies have been detected in the blood of AIDS patients^{1,2} and form the starting point for the production of vaccines³. However, there are as yet no indications that these antibodies influence the course or the outcome of the disease. Therefore, alternative strategies should be pursued. A recently developed technique for specific inhibition of gene expression provides one possible new way to tackle the AIDS virus. It is based on the inhibition of the messenger RNA translation through the interaction of anti-sense RNA with a specific messenger and in several systems has been shown to be very successful^{4,5}. Accordingly, the introduction of an actively transcribed template for anti-sense RNA of one of the AIDS virus genes into infected cells may induce inhibition of virus replication and increase survival chances of the patient.

The construction of such a template will not be difficult, but how is it to be delivered to the correct target cells? The answer is, in principle, simple. Make a recombinant AIDS virus in which one of the genes is in the reversed orientation. Infection of a patient with this virus will ensure that the recombinant genome is delivered precisely to those cells that are sensitive to, or harbour, the AIDS virus. Cells not yet invaded by the AIDS virus may be protected by the recombinant anti-sense RNA against integration of the AIDS genome. In this way spreading of the virus within the body could be prevented. Which segment of the genome of the virus should be reversed? The *gag* or *pol* regions are unattractive, because the corresponding genes of any helper virus involved in the production of the recombinant might be blocked as well. With the reversion of the *env* gene there is the additional problem that the recombinant virus might acquire the envelope of the helper, resulting in the infection of the wrong target cells. A fourth gene, *tat*, which has recently been identified on the genome of the AIDS virus^{7,8}, is more promising. The gene product is a trans-activator of viral transcription. Preventing the synthesis of the *tat* protein results in a significant reduction of virus replication, which is exactly what we are looking for. In fact, reversion of the second exon of the *tat* gene, which is located between *pol* and *env*, would alone be sufficient.

In theory, the only problem that we are left with is the efficient production of the recombinant virus, because this may require the action of the *trans*-activator protein. A cell line producing and accumulating the *tat* protein in sufficient quantity, prior to infection with the recombinant

virus, might be effective. Now that the sequence of the gene is known such a cell line is within reach. Alternative methods can be thought of, one of which is to load cells before infection with the *tat*-protein, obtained from any source, by the method of scrape loading⁹, which works well especially with proteins of small size.

Although problems would surely show up during the development of the strategy outlined above, AIDS is too serious a threat to human life to let any possible means for therapy and prophylaxis go untested. The method might also be tried for other diseases, including the T-cell leukaemias caused by HTLV-I and -II.

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SIR—We wish to suggest a gene therapy scheme for the control of acquired immune deficiency syndrome (AIDS). The current view on AIDS is that infection with HTLV-III/LAV virus, tropic for OKT4⁺ lymphocytes, results in breaking the backbone of the immune system. The diversity of viral strains and the nature of the infection renders problematic the production and use of vaccines¹. A strategy has been proposed consisting of "protecting" T-cell clones against HTLV-III by using viral interference through infection with attenuated virus².

In our view a better way to achieve this protection would be through the strategy of antisense RNA. Inhibition of gene expression has been achieved in a variety of systems with as little as 52-base-pair, homology to the 5' untranslated segment of the sense RNA³. Helper-free, defective HTLV-III, containing the appropriate group-specific inverted viral gene segments could be produced in a packaging cell line modelled on the Zipneo-ψ2 system⁴. To ensure out-titration of sense-RNA, a gene amplification system, such as the DHFR gene responsive to the anticancer drug methotrexate, could be built into this antisense virus⁵.

AIDS therapy would consist in removal of bone marrow stem cells from patients, followed by their infection with the anti-virus and reinsertion, resulting in the production of "protected" lymphocytes. Alternatively, peripheral blood lymphocytes obtained by pheresis would be infected and reinserted. Methotrexate induced amplification of the antisense provirus may be carried out in vitro, as needed⁶. By these means a critical and sufficient population of T cells might be protected so that an effective immune

response against all strains of HTLV-III and against opportunistic infections could be achieved.

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Cerebral supermanifolds and little brains

SIR—John Marshall's analysis of current neuropsychological efforts to correlate specific lexical dysfunctions with "focal" brain damage raises, expectedly, some presently unfocused issues: (1) The "focal" brain damage reported by Hart *et al.*¹ was a "left-hemisphere cerebrovascular accident" diagnosed by computerized tomography as "an infarction involving the left frontal-lobe and basal ganglia." This is, by cerebral neuroanatomical criteria, a truly enormous, almost global region of damage.

(2) Marshall's interesting quotation from Johann Gesner² suggests that even the classical eighteenth century allowed speculation about the validity of linear transformations from a 'psycho'-logical to a cartesian frame of reference in cerebral cortex.

(3) Despite some rough approximations to a two-dimensional somatotopy found in mammalian and primate somatosensory-specific cortex (for example, the primate digit and mouse whisker-barrel "representations"), we have no reason to suppose a linear two- or three-dimensional mapping of inner and outer spacetime to exist inside our heads. Since the days of Maxwell to Einstein would it not be more reasonable to use differential geometry in our approach to understanding how brains might work? A start has already been made for our "little brains".

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Scientific Correspondence

Scientific Correspondence is intended to provide a forum in which readers may raise points of a rather technical character which are not provoked by articles or letters previously published (where the Matters Arising section remains appropriate).



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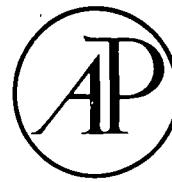


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Feathered wisdom

Freeman J. Dyson

Hawks, Doves and Owls: An Agenda for Avoiding Nuclear War.

Edited by Graham T. Allison, Albert Carnesale and Joseph S. Nye, Jr.
W. W. Norton: 1985. Pp. 282. \$14.95, £12.95.

THIS book has been edited by three pillars of the Harvard establishment who have long experience as expert advisors to the American government. They have produced an introductory chapter and two summarizing chapters. In between are six chapters written by others, discussing in greater depth six particular contexts within which nuclear war might arise. Each is a scholarly analysis, full of historical facts and supported by numerous footnotes. The book as a whole is readable and tells a coherent story without becoming repetitious. Its main emphasis is put on confusion as the most likely cause of nuclear war.

The longest chapter is also the most informative. It is "Soviet Perspectives on the Paths to Nuclear War," by Stephen Meyer, director of the Soviet Security Studies Working Group at MIT. Unusually, for an American discussing strategic questions, Meyer has paid careful attention to what the Soviet military authorities say and do. Half his extensive footnotes refer to Russian-language sources. But more than this he says: "Let me begin, then, by noting some sources not used in this chapter. First in this category are official Soviet pronouncements on military doctrine and policy . . . A second source not used is the work of Soviet academics . . . This chapter's sources include Soviet military planning literature, Soviet military historical analyses, Soviet diplomatic behavior, Soviet military force structure and deployment data, and memoir material." Most of us amateurs who claim to know something about Soviet attitudes collect our impressions from the two sources which Meyer discards as unreliable. His conclusions are firmly based and not proffered where evidence is lacking.

Meyer's description of Soviet attitudes emphasizes the Soviet doctrine of pre-emption. He does not overlook the inconsistencies between the formal 'no-first-use' policy enunciated by Brezhnev and the practical reliance on pre-emption which is visible in Soviet operational planning. The inconsistencies will not be quickly or easily resolved. We see a clear recognition by the Soviet military that decisions on the use of nuclear weapons are restricted to the supreme political authorities, and we see military plans which take the political decisions for granted.

Meyer believes that in an acute crisis the military plans are likely to prevail. The

most serious danger of nuclear war would arise from a Soviet decision to launch a pre-emptive attack in response to a sudden and panicky mobilization of NATO forces at a moment of crisis. He regards as particularly unwise the American habit of putting nuclear forces on world-wide alert as a signal of political resolve. This was done during the Cuban crisis of 1962 and the Egyptian crisis of 1973, in both cases without causing disaster. If it is done again in a future crisis arising closer to Soviet territory and appearing to threaten Soviet vital interests, disaster may be more difficult to avert.

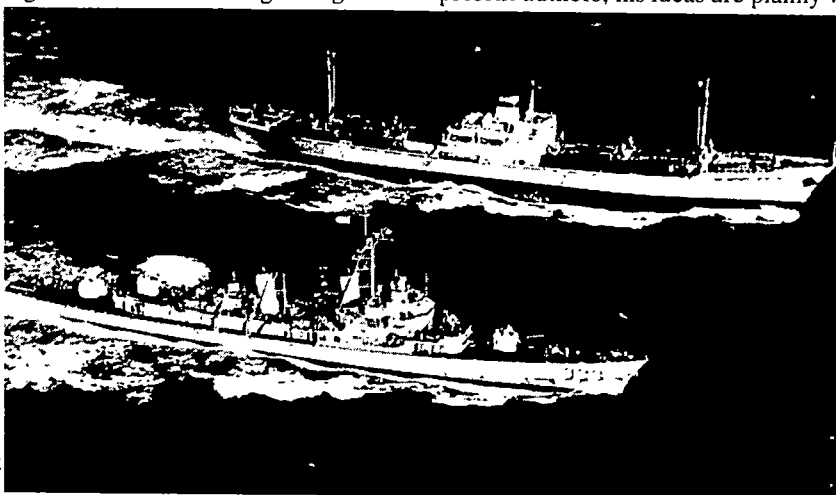
Another good chapter is "Escalation in Europe" by Fen Osler Hampson, a Harvard post-doctoral fellow. One of Hampson's important suggestions is that, in case of serious fighting in Europe or in the Middle East, the escalation to nuclear war may happen more easily at sea than on land. Naval units on both sides have a freer access to nuclear weapons than land units, and the political controls at sea are looser. At sea there is an abundance of tempting targets for nuclear weapons, and the absence of civilian populations from the battlefield makes it easier for the warriors to yield to temptation. For all these reasons, the nuclear weapons at sea are more likely than those on land to get us into an unintended nuclear war.

There is an outstanding opportunity for us here to lessen the risk of nuclear war by extending and reinforcing the American-Soviet Incidents-at-Sea Agreement of 1972. The Incidents-at-Sea Agreement has two main components. It formulated an agreed set of rules for regulating traffic

in areas such as the Mediterranean where the two navies are apt to encounter each other frequently. And it established a joint commission of American and Soviet naval officers who meet twice a year to discuss dangerous incidents and to revise the rules when necessary. The holding of regular and quiet meetings between military officers is perhaps the most useful immediate step we can take to decrease the probability that local crises will escalate to general war. There is no reason why similar meetings should not be held by armies and air-forces as well as by navies.

The final summary by the editors is divided into two parts. First they give us an analytical framework for thinking about nuclear strategy; then they tell us what to do. The framework is based on the three ways in which people are afraid that a nuclear war might begin. War might begin because national leaders are weak and irresolute in standing up to aggression, or because they are paranoid in reacting to imagined or exaggerated threats, or because they are confused and unable to control events at moments of international turmoil. People who are most afraid of weakness are hawks. People who are most afraid of paranoia are doves. People who are most afraid of confusion are owls. The editors remark that all three fears are well justified by the evidence of past history and present realities. Hawks, doves and owls are all speaking truth.

A rational strategy must deal with all three fears simultaneously. What to do, is a question of balance and emphasis between the three dimensions of danger. The debate until now has been largely between hawks and doves, and has for that reason become ideological and impractical. The editors seek to redress the balance by giving major emphasis to the concerns of the owls. One of the wisest of the owls is William Ury, whose recent book *Beyond the Hot Line* gave us a number of sensible suggestions to mitigate the dangers of war arising from accident, incompetence and panic. Although Ury is not one of the present authors, his ideas are plainly visi-



High tension — the USS Barry steams alongside the Soviet freighter Anosov off Puerto Rico during the Cuba crisis of 1962. Could we now cope better in such a situation?

ble in many of the chapters. He was a member of the Avoiding Nuclear War Project out of which the book grew.

The final list of recommendations is divided into ten sections. Two of the sections are hawkish, two are dovish and six are owlsh. These proportions reflect the editors' belief that at present the dangers of war through weakness or paranoia are over-rated and the dangers of war through muddle are under-rated. The majority of their recommendations are directed toward avoiding a nuclear version of the outbreak of war in 1914, an outbreak driven by geographical and historical accident rather than by any grand design.

A small sample may indicate the general flavour of the editors' recommendations. I choose five that I consider characteristic, one hawkish, "Don't adopt a No-First-Use policy." one dovish, "Don't assume that nuclear deterrence will last forever," and three owlsh, "Do reduce

reliance on short-range theater nuclear weapons, do work with the Soviets to prevent and manage crisis, do encourage non-governmental contact with the Soviets." It is not necessary to agree entirely in order to applaud the clarity of their thinking and the brevity of their style.

I happen to disagree strongly with their No-First-Use recommendation, and still I recommend their book whole-heartedly as a fair and lucid statement of the nuclear dilemma. Only one essential thing is absent from their world-view. They lack a sense of the absurd. It may be that a resolution of the nuclear dilemma will come in the end from a general recognition of its absurdity. I would like to add one more recommendation to their list, "Don't take nuclear weapons more seriously than they deserve." □

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Manual works

Graham Richards

A Handbook of Computational Chemistry: A Practical Guide to Chemical Structure and Energy Calculations. By Tim Clark. Wiley: 1985. Pp.332. £35.80, \$35.

Semi-Empirical Methods of Quantum Chemistry. By Joanna Sadlej. Ellis Horwood: 1985. Pp.386. £47.50, \$71.25.

COMPUTATIONAL chemistry has come out of the closet. No longer is the calculation of molecular conformational energy or electronic distribution confined to a small clique of tolerated but mildly deviant academics who scorn conventional experimental practice. Even in the staid halls of industry, theoreticians are now to be found working alongside straight organic chemists, designing novel compounds with the aid of calculations — both quantum mechanical and of the molecular mechanics type — and, increasingly, assisted by sophisticated computer graphics displays.

This growing army has a real need for a handbook which details not the theory behind the methods used, but rather where to start and how to use the program packages available. The most favoured weapons currently in use for elucidating conformation are programs based on molecular mechanics (treating molecules as balls and springs with empirical force fields). These are supplemented with molecular orbital calculations to unravel the electronic details, with the semi-empirical methods known by the acronymic titles MINDO, MNDO and MOPAC becoming standard, together with variants on the *ab initio* molecular orbital method developed by Pople and his co-workers (currently known as GAUSSIAN 82).

The use of all these packages requires

the chemist to present as input the geometrical structure of the molecule under consideration. Clark's excellent manual provides clear and detailed instructions on how to submit data for the most widely adopted packages. It is replete with examples of sample input and of output, just the thing to enable an experimental chemist to be able to use the available programs. The book does not provide much about the general background theory, nor does it underline the rather shaky theoretical basis for some of the methods (these matters are, however, discussed in many, more conventional textbooks on theoretical chemistry). Instead we have a very practical manual, the review copy of which has already been tested by novice graduate students with happy results. The theoretical section of chemistry bookshelves is generally overstocked, but this book fills a genuine gap and will undoubtedly be found at the elbow of many users of packages.

Sadlej's text is in an overlapping area and is a more conventional account of the semi-empirical methods of quantum chemistry: sadly it is the book one wanted ten years ago. The Polish edition was written in 1976 and although "revised", the age shows. The bibliography goes up only to 1978, and the techniques upon which the book concentrates — the Hückel method and CNDO — are both now somewhat *passé*.

Those theoretical chemists who resent the loss of their exclusive and even furtive world will no doubt grumble that running a computer package no more makes one a theoretician than sleeping in a garage makes one a mechanic. But computational chemistry is on the rise and life should be made easier for the virgins. □

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Cool side of life

Pierre Douzou

Biophysics and Biochemistry at Low Temperatures. By Felix Franks. Cambridge University Press: 1985. Pp.210. £25, \$44.50.

ALTHOUGH Felix Franks in a sense is correct in his forward "cold is the fiercest enemy of many forms of life", biologists dream of exploiting it for the cryopreservation of "spare parts" of living systems amongst other problems. Like physicists some decades ago, biologists are becoming increasingly interested in the measurement, interpretation and exploitation of natural phenomena at low temperatures, and cryogenic research is slowly becoming a branch of this discipline. Franks' book is a step along the way in this endeavour.

The book gives clear answers to the classical questions about the physics and physical chemistry of water at sub-zero temperatures, the effects of its behaviour on life, and current applications in biochemistry. Other specialized chapters survey our understanding of the responses of the cell and of living organisms to cold, and of the problems of their preservation in the laboratory, a problem still dominated by empiricism and clouded by the interplay of an extraordinary variety of factors. The book ends with a survey of future prospects in this challenging but promising field.

Franks has been a leading contributor to research on water structure and properties, here he combines this expert insight with his interest and recent experience in cryopreservation for the task of explaining what is known and what is uncertain, and of suggesting what can be done to set up a useful cryobiology. The writing of a book on this topic demands a grasp of many specialized fields and is a daunting task, but the result here is agreeable and very instructive, because the book reveals its origin as a lecture course by a man trained as a physical scientist who drifted into the life sciences "fairly late in life and [who] never received any formal teaching in biological dogma" and had first to teach himself.

The book gives a strong impression of unity, carries with it a tone of optimism and in some cases of certitude and is valuable in that it covers a range of subject material which has never before been brought together in such an organized and concise yet clear manner. It will be of great interest to that growing band of researchers working on the cryobehaviour of living organisms. □

Pierre Douzou is Professor at the Museum d'Histoire Naturelle, Paris and head of Department at the Institut de Biologie Physico-chimique, 13 rue P.M. Curie; 75005 Paris, France.

To win friends and influence people

Ian Stewart

The Elements of Graphing Data. By William S. Cleveland. *Wadsworth:1985.* Pp.323. Hbk \$27.95, £37.80; pbk \$18.95, £25.60.

"In 1980," says the author, "I began a study of graphs in scientific publications . . . In one study, I read the articles and reports of the 1980 Volume 207 of *Science* . . . 30% of the graphs in the volume had at least one of four types of specific problems." Those problems were: something on the graph but not explained, parts of the graph being visually indistinguishable, and mistakes such as mislabelling or poor reproduction. Deeper problems included poor choice of the form in which to present the data, and indeed poor choice of data. In an effort to combat these problems, and to suggest effective counter-measures, this book discusses the principles and methods of graphical construction, amply illustrated by examples selected from the literature (with references, which may lose the author a few friends but makes the arguments more cogent).

Scientists spend a large part of their training learning how to do science; and very little learning how to communicate it. Perhaps this is unavoidable, especially given the pressures of crowded curricula; possibly it would make little difference if students attended the odd course on good presentation and effective data display. But, at the very least, they could be encouraged to read a book such as this. Once sensitized to the pitfalls, they might be more prepared to put in the effort required to select a clear and effective means of presenting their results.

Consider even the most standard type of graph, with two axes, some tick marks

to show the scale, dots for the data, and lines connecting the dots. Do the data stand out, or are they obscured by the connecting lines? Do the data points overlap and obscure each other? Do the axes fall across data points? Are there too many tick marks? Do the labels clutter the picture and make it hard to grasp the significant features of the data? All these matters require proper attention. Other types of graphical medium, such as the scatterplot, pose other questions.

One especially fraught area is the display of multidimensional data. To graph the relations between ten or twelve different variables can stretch even the most active imagination. Most attempts fail in one way or another. The author suggests the use of colour to improve readability; this is fine in principle, but most journals refuse to include colour because of its cost. The scatterplot matrix — an array of coordinated plots of all possible pairs of variables, one against the other — manages to convey a great deal of information succinctly and clearly.

The danger with principles is that they can be taken too literally. A few horrific examples show what happens when the Darrell Huff Dictum, "always include zero in the scale", is overdone. To cramp the data into a tiny corner of an otherwise blank graph helps nobody. Huff was thinking more about advertising or political propaganda, and when preparing a graph for scientific publication, it should be permissible to assume that the reader has the intelligence to read the numbers on the axes.

If you're not willing to present your data effectively, then presumably you don't really care what they are. If you don't write papers that others can read, why write papers at all? The author's basic message is a simple one: remember that the important thing is the data. Granted this, all else will follow. □

Ian Stewart is a Reader in Mathematics at the University of Warwick, Coventry, CV4 7AL, UK.

A look around the kitchens

Alastair Hay

Controlling Chemicals: The Politics of Regulation in Europe and the United States. By Ronald Brickman, Sheila Jasanoﬀ and Thomas Ilgen. *Cornell University Press:1985.* Pp.344. \$38.45.

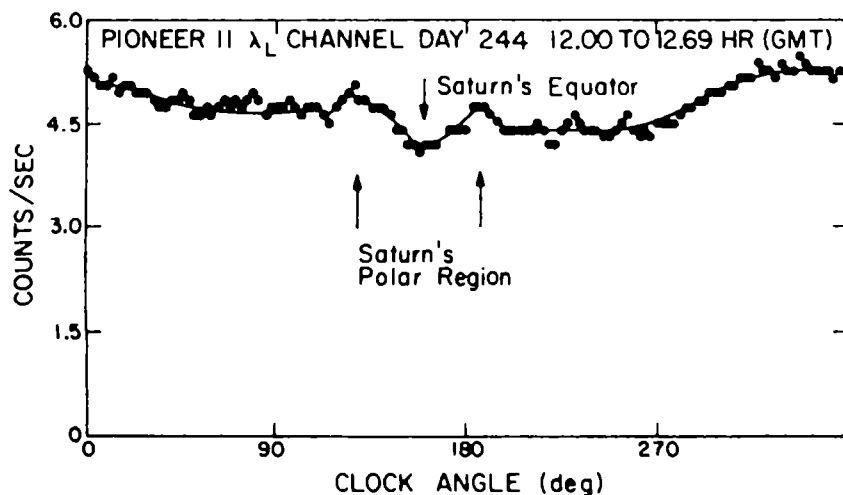
EARLY attempts by some United States Government Agencies to control the availability and use of chemicals were derided by critics, and the chemical industry in particular, as "cook-book" approaches to the subject. Fifteen years later things have changed, but analogy with cooking remains an appropriate way to describe the thesis of this book.

The authors have set out to compare how chemicals are regulated in Britain, West Germany, France and the United States. It is as if four cooks were asked to bake a loaf of bread — starting with the same ingredients, they follow different recipes but end up with a loaf nevertheless. The European cooks keep their techniques to themselves, arguing that their success lies in some secret mix. The American, on the other hand, has an audience to scrutinize all the ingredients, check the recipe and follow each step from mixing to baking.

The same applies to chemical regulation. Europeans prefer a consensus approach, negotiating with interested parties behind closed doors and presenting final versions of regulations which are rarely altered by Parliament. In the United States, however, the procedure is adversarial, with the government, Congress, the courts, public interest groups and industry all having a say in the matter.

While recognizing that the European pattern of involvement is more restrictive, the contention of Brickman and his colleagues is that, despite this lengthy and expensive process, legislation in Europe is as — or more — effective as that in the United States. (To prove the point, a comparative table of regulated substances is appended to the book, showing limits for the four countries.) Their conclusion is drawn from a thorough analysis of the European systems, some features of which, they argue, could well bear consideration by both regulators and legislators in the United States.

There are fundamental differences between the regulatory processes applied in Europe and the United States. Where the scientific evidence on the hazards — and the carcinogenic risk in particular — of a chemical is unclear, the Europeans tend to adopt a policy of 'innocent until proven guilty'. In the United States, however, guilt is assumed, until proven otherwise, and decisions about regulations are made from that standpoint.



The resolution of this graph is degraded by the inclusion of zero on the vertical scale.

Brickman *et al* point out that "concepts like 'reasonable' and 'practicable' sum up British regulatory philosophy, a philosophy generally shared by industry". Discussions about what these terms mean as far as particular chemicals are concerned are carried out in private between civil servants and industry. In the case of decisions taken by Britain's Health and Safety Executive, workers' trades unions are also involved. The result, say the authors, is usually a compromise that all sides are prepared to live with.

On the question of whether there is a 'safe' concentration for carcinogens to which workers can be exposed without risk, Britain seems to be out of step. British regulators, are favourably disposed to the concept of a threshold. As pointed out in the book, a guidance note issued by the British Health and Safety Executive in 1978, proposed a method of determining a "practical threshold of neoplastic response" while conceding that a "precisely defined" threshold cannot be attained. But Britain, it seems, is the only country that has banned some chemicals in the workplace because of their carcinogenic properties.

In Germany, the analysis of hazards is usually carried out by scientific advisory committees within each Ministry, who then also support appropriate regulations. It appears that these committees have a similar philosophy to the US Agencies in that they accept that there is no safe

threshold level for carcinogens. Because the German Commission on hazardous substances in the workplace argues that there is no way of assessing a threshold and calculating a tolerance level, this leaves the Federal Labour Ministry free to set exposure standards for carcinogens at any level it regards as economically and technically feasible. Similarly, the French are reluctant to establish threshold limiting values, and the strategy for regulating carcinogens is to "combine strict medical monitoring with reporting and personal hygiene requirements".

In the United States, however, legislation is preferred. But the strict standards for chemicals in the workplace recommended initially by Regulatory Agencies are invariably toned down by a process of attrition in the courts. The end result of this is standards similar to those in force in Europe.

While the author's principal conclusion is the need in the United States for a more uniform approach to rule-making, and an opportunity for more negotiations between competing interests, they do not envisage less information on chemical hazards being released to the public (something which would win widespread approval in Europe). It is ironic that at the same time that some Americans are recognizing the benefits of toning down their procedures, many Europeans are starting to see merit in a system of greater accountability — that is, they would like more people to be let into the kitchen. This well-researched and well-argued book will be an excellent starting point for people of both persuasions. □

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Get together

Marie Boas Hall

Science Reorganized: Scientific Societies in the Eighteenth Century. By James E. McClellan III. *Columbia University Press*. 1985. Pp.413. \$38.50, £41.80.

To join with others in a more or less formal group seems a natural human instinct of modern society. European scholars have long done so, in forms conditioned by the state of society: in the Renaissance under noble or princely patrons, later as a subsidiary of centralized monarchies, later still as a manifestation and a reflection of emergent nation-states. The first resultant academies were mainly literary or musical or scholarly; as science developed in the seventeenth century its practitioners drew together, either by correspondence or by association, and scientific academies, institutes and societies were born. And it is the societies and organizations themselves that form the focus of Dr McClellan's book.

Of these emergent academies the Royal Society of London (founded 1660, chartered 1662) and the Académie Royale des Sciences of Paris (1666) are the oldest in continuous existence, although not the first to be conceived. Both differed from earlier foundations in being independent of a private patron, in being incorporated entities and in publicizing the work of their members. The Académie (itself parallel to the Académie Française of 1635) was the prototype of modern national institutes and academies, its members paid by and sometimes appointed by the state, while the Royal Society was and is independent of the Government, though adviser to it and an agent of it.

Until roughly 1730 the Royal Society had the more eminent members and hence the greater reputation; slowly, in the wake

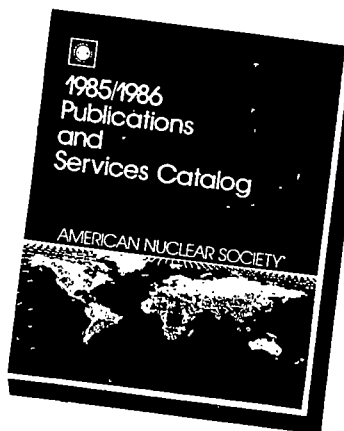
of reform in 1699, the Académie reversed the situation. Because of this and the fact that French culture and politics dominated eighteenth-century life in continental Europe, later academies were imitations of that of France, until Berlin, St Petersburg (both initially mainly consisting of foreign savants), Uppsala, Turin and a host of provincial cities all possessed formal organizations with charters, publications and paid members. These were sometimes purely scientific, sometimes, especially later in the century with growing linguistic nationalism, combining a scientific with a parallel literary section, while England, after the growth of private provincial societies, about 1790 saw the rise of the "Lit. and Phil." movement.

What was the cause of all this activity? Dr McClellan as he surveys the eighteenth-century scientific academy sees it as the result of increasing professionalism in science (he does not discuss non-scientific groupings). He is interested in the organizations, which he describes and analyzes fully, not in the individuals who made them up nor, except where cooperation between societies was involved, in the work they accomplished. This gives a lack of solidity to his account, reinforced by a certain naivety about European (including English) history, pointed up by the American sections. (Nor has he a very sharp grasp of language.) He is at his best in describing the structure of the new continental European academies and the relationships between them and he rightly regards as innovative the beginning of a formal (as distinct from a personal) exchange of publications begun in mid-century. There is a very great deal of information in this book which clearly shows how scientific academies developed during the eighteenth century. □

Marie Boas Hall, 14 Ball Lane, Tackley, Oxford OX5 3AG is Emeritus Reader in History of Science and Technology of the University of London.

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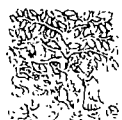
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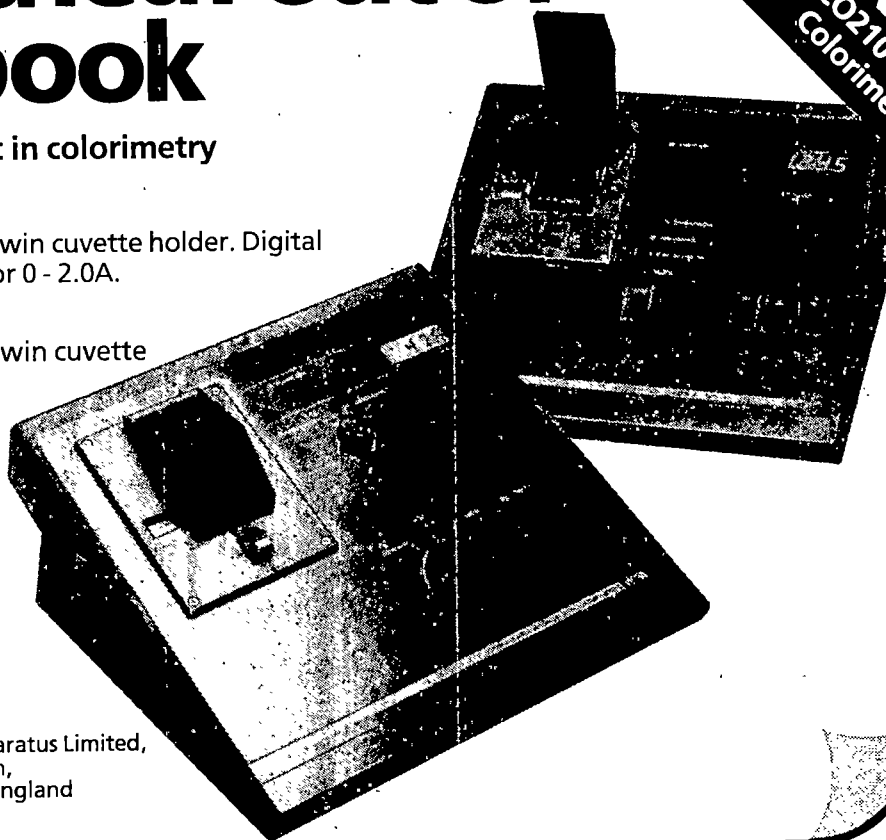
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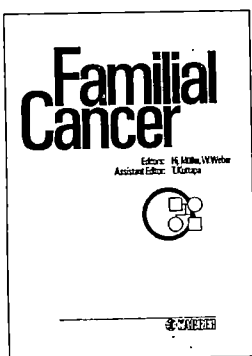
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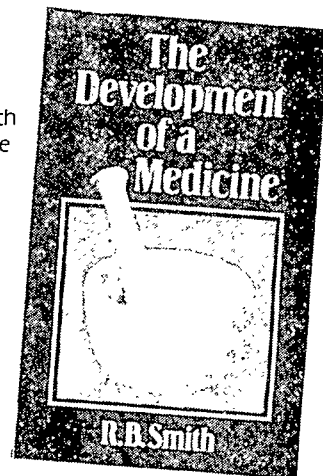
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Part 2. The participating astrologers were separately given the natal chart of a random subject and an objective and respected measure of his personality traits called the California Personality Inventory (CPI). They were also given two other

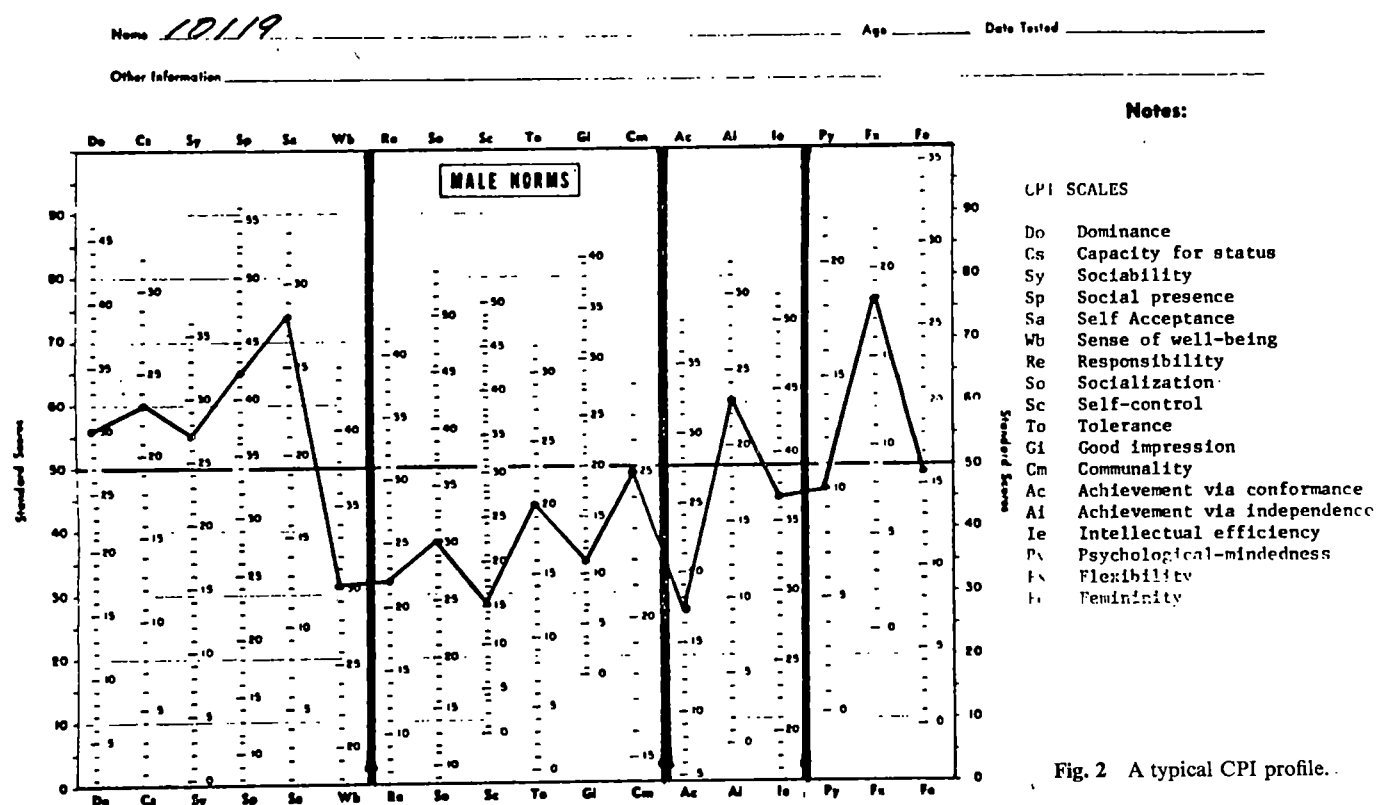


Fig. 2 A typical CPI profile.

CPIs chosen at random from the group of all the subjects' CPI test results. The astrologers were then asked to select the two CPIs (first and second choice, no ties allowed) which described personalities closest to the personality indicated by the natal chart. They also rated each CPI on a 1-10 scale (10 being highest) as to how closely its description of the subject's personality matched the personality description derived from the natal chart. The scientific hypothesis predicts a correct choice one third of the time; the astrologers predicted a correct choice half the time or more.

The two parts of the experiment are complementary; the first is not sensitive to biases in the CPI, the second does not make the possibly false assumption that subjects can accurately judge their own personalities. We decided at the outset to require a 2.5 standard-deviation increase over random chance to interpret the results as favouring the astrological hypothesis. Similarly, a disagreement by 2.5 standard-deviations or more would be required to reject the astrological hypothesis in favour of the scientific one. Otherwise, we decided in advance, we would draw no conclusions of significance. No data were analysed until all the data had been collected.

Experiment design

To eliminate bias, both anticipated and unknown, we made extensive use of double blind techniques. All subjects were assigned a five-digit random code number. Neither the astrologers nor the experimenter knew what code number corresponded

to which person. These lists were solely under the supervision of Richard A. Muller, Professor of Physics at the University of California, Berkeley.

Guidance was sought both from the scientific and astrological communities. To help ensure correctness of the testing method and statistical analysis, the scientific adviser was Professor Muller. So that participating astrologers should be respected by the astrological community, we sought the advice of the National Council for Geocosmic Research (NCGR), an organization which has been involved in much astrological research in the past and which has the respect of astrologers world wide; NCGR nominated persons who consented to be our astrological advisers, and who carefully reviewed the experimental design and made many suggestions.

After they were satisfied that the experiment was a 'fair test' of astrology, our astrological advisers established their predictions (50 per cent for both part one and part two) as the minimum effect they would expect to see. They also compiled a list of approximately 90 astrologers with some background in psychology who were familiar with the CPI and held in high esteem by their peers. It was the opinion of the advisory astrologers that a random sample from this list would be able to score at the predicted 50 per cent level. All were invited to participate; 28 accepted. (Only two astrologers who participated were not on the original list. They heard of the experiment and wanted to take part. After their qualifications had been vouched for by NCGR, they were admitted.)

Constructing a natal chart is a simple but laborious mathematical process, so computers are well suited for the task; several machines designed specifically for this purpose are available on the market. To save time and ensure accuracy, all natal charts were constructed by Mr. Caveney (President of the San Francisco chapter of NCGR) and Mr. Nelson (Secretary of the San Francisco chapter) on a Digicomp DR 70 Astrological Computer; they were spot-checked by hand calculation.

The California Personality Inventory (CPI) is a standard personality test¹⁻³ which has been used extensively since 1958. It was chosen over other available personality tests because the advising astrologers judged the CPI attributes to be closest to those discernable by astrology. By choosing this test we were thus trying to maximize the ability of the astrologers to match CPI data with natal charts without introducing a pro-astrology bias. Other experiments have been done using the CPI with apparently positive results⁴.

The CPI consists of 480 true-false questions, each of which helps to rank a subject on one of 18 personality attribute scales (for example, dominance, passivity, femininity, masculinity). The subject's score on each scale is compared to the norm for that scale. The scores can be plotted on a graph (see Fig. 2) which readily conveys this information. Such a graph is called a 'CPI profile'.

Personality tests were graded, after names had been replaced by code numbers, by volunteers (undergraduate students) who were in no other way connected with the experiment. From spot

checks of the grading, we determined (95 per cent confidence level) that mistakes by the graders contribute an error of more than two points to CPI scores on fewer than 2.6 per cent of the individual scores, an insignificant effect.

Subjects were solicited by advertisements in San Francisco Bay area newspapers, classroom announcements and postings on and off the Berkeley campus. (To protect the confidentiality of the data and the rights of subjects, all procedures were checked by the University of California Office of Fair Treatment to Human Subjects before beginning data collection.) Approximately 70 per cent of the subjects were college students and about one half of these were graduates. All subjects were required to fill out a questionnaire with their natal data (birthday, including exact time and location of birth). They were also asked whether they (1) believe in astrology, (2) believe somewhat, (3) have no opinion, (4) disbelieve or (5) strongly disbelieve in astrology, and to state whether they had ever had a natal chart constructed before. Subjects were not told that these questions affected subject selection, but those who chose "(5) strongly disbelieve" were eliminated, on the grounds that this opinion might bias them, either consciously or unconsciously, against selecting the interpretation which best fitted them. All those who had previously had a chart constructed were similarly eliminated because they might be able to select (or reject) the correct interpretation based on a knowledge of what to expect. Strong believers who had never had their charts done were, however, not eliminated; this belief alone could not help them select the correct interpretation. All subjects had to be at least 17 years old. Failure to take the CPI resulted in rejection.

To avoid the possibility that a subject may have had his natal chart constructed elsewhere, or may have changed his opinion about astrology, between the time he submitted his natal data and the time he was given the final natal interpretations (typically 8–10 weeks), all subjects were required to fill out a new questionnaire before being asked to choose their own natal interpretations in Part 1 of the experiment. Two subjects were eliminated at this point, one who admitted to being a professional astrologer (and who had apparently lied on the first questionnaire) and another whose opinion of astrology had changed from "disbelieve in astrology" to "strongly disbelieve in astrology".

We encouraged prospective subjects to participate by promising them a copy of their natal chart, CPI test results and interpretation, the completed natal interpretation, and a copy of the final results of the experiment.

Although we required a departure from random of only 2.5 standard deviations to interpret the results as favouring the astro-

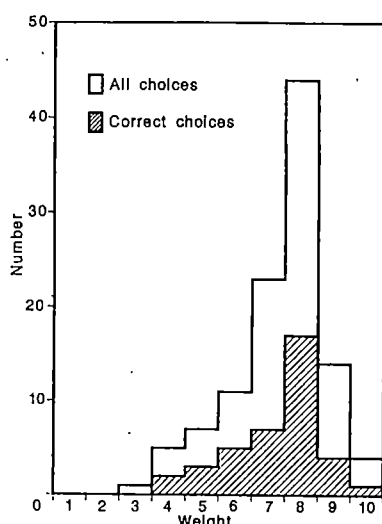


Fig. 3 Histogram showing weights assigned by astrologers to the CPI profiles they felt best fit the natal charts. CPI profiles rated higher are not more likely to be correct.

logical thesis, we originally planned to be able to distinguish between the two hypotheses at the four standard deviation level. This is why the number of subjects required was chosen to be 128, with a further 128 as a control group. Thus the total number of subjects was originally 256, but many of these did not complete all phases of the experiment.

Many lost interest and did not return their data to us. Some moved in the time taken to send them the test materials and did not leave a forwarding address. Two room-mates became emphatically convinced that astrology was the work of the devil, and refused to continue in what they called 'an experimental test of evil'. We were forced also to eliminate 12 subjects either because they did not follow directions correctly or did not return all the needed materials to us.

The use of double blind techniques is most important during this stage of the experiment. During the process of rejection of data, the experimenter had no access to any information that might introduce bias. In the end, only 177 subjects (83 test group, 94 control) remained for Part 1 of the experiment. Neither were we able to collect all the data we had hoped to in Part 2 of the experiment. First, fewer astrologers than hoped for agreed to participate. 224 data envelopes were mailed to only 28 astrologers, some of whom simply refused to participate as promised. Some declined after they discovered how much time was required on their part. One tried to bargain his services in exchange for free access to our raw data, and declined to participate when his terms were refused. For these reasons, we obtained only 116 usable subjects for Part 2 of the experiment. The large reduction in numbers was unanticipated and reduced the expected discrimination between hypotheses for Part 1 to 3.2 stan-

dard deviations, and for Part 2 to 3.9 standard deviations. However we do not believe that the loss of data could bias the results of the experiment in any significant way.

Bias and control

Experiments using human subjects are subject to a special class of biases which do not normally have to be considered by a physical scientist. An experiment must be designed so that the psychology of the subjects will not alter the results. The major potential biases which required specific control in the experiment design were as follows:

Sun-sign bias. By the astrological definition, the 'Sun-sign' refers to the constellation of the zodiac in which the Sun resides when the person is born. If the Sun-sign should play an important role in the average chart and if people are generally familiar with the characteristics of their Sun-sign (through newspaper horoscopes, for example), we might expect them to select the correct interpretation at a better-than-chance level regardless of whether or not the astrological hypothesis is correct.

To correct for this, each member of the test group was matched to a member of the control group born under the same Sun-sign. Following the astrologers' recommendations, we required that the age difference between these subjects be at least three years, so that their natal charts would be 'sufficiently dissimilar', but otherwise, the assignment was made randomly. Both test and control subjects were given the same three interpretations.

If the astrological hypothesis is false, members of both groups should identify the test subject's interpretation with equal frequency. If the hypothesis is true, the test group should score significantly higher than the control.

We believe that many other sources of bias are eliminated by the design of the experiments and by the standard formats in which data was conveyed to and from the astrologers. The possibility that subjects (in Part 1) would be tempted to choose flattering interpretations (or the opposite) should not bias our conclusions, given that we are comparing hits and misses between test and control groups. We sought to eliminate from the interpretations clues to their origin that might guide a subject to a correct choice, such as astrological terms that might be part of subjects' general knowledge, and to eliminate from the data with which astrologers worked, information that might allow them, consciously or otherwise, to bury hidden clues in their interpretations. Thus we eliminated from the charts transmitted to the astrologers information about the time and place at which subjects had been born. (Although the charts in principle allow the reconstruction of this information, the process is time-consuming, and the outcomes unlikely to be of direct help to

astrologers.) We did not tell the astrologers of the gender of the subjects, partly because gender-specific elements of the interpretations might lead to bias, but also because the CPI scores cover different ranges for males and females.

To eliminate the possibility that subjects could pick up clues other than the astrological information we were testing, and to insure that the information given to subjects was as uniform, and thus as comparable as possible, the interpretations followed a predetermined format designed to specify what factors astrologers should derive from the chart and to set a limit on the length of written material. The format was developed in collaboration with the advising astrologers. The specific categories which astrologers were required to address were: (1) Personality/temperament; (2) relationships; (3) education; (4) career/goals; and (5) current situation. The astrologers typed each interpretation on pages supplied by the experimenter and containing the proper headings, again to keep the interpretations as uniform as possible.

The format also specifies: (1) that advice or predictions were not to be given, on the grounds that such information could not help the subject to select the correct interpretation but that it might well lead him to discard an accurate description because he disagrees with the advice or predictions given. (2) That no direct reference to the chart was to be made (e.g. "you have sun in Leo"). (3) That no information relating to the subjects' ages was to be given.

Subjects were asked to rate each section of each natal interpretation on a 1-10 scale. Then they were asked to write down, for each section, the code number of the interpretation which fitted them best and second best.

One complication of our study arises because a subject's ability to select the correct description of himself from a given group must depend on how well he knows himself. If people generally have an inaccurate self-image, one would not expect subjects to select the correct interpretation no matter how accurate are the results of astrology. We devised the following scheme to understand this potential bias.

The CPI is generally accepted by psychologists as a moderately accurate description of a person's personality. Each test subject was given his own CPI profile and two others randomly selected from the group. He was then asked to select the profile which he felt best fitted him. Each subject was provided with the following: (1) three sample CPI profiles; (2) a synopsis of what high and low scores in each category tend to be for males and females; (3) a letter explaining about a CPI profile and how to go about making the selection.

To control for possible psychological bias, we elected to use the same test and control groups as in Part 1, but since the CPI is graded on different scales for males and females, we had to match male

Scientists wishing to familiarize themselves with astrology as astrologers practise it may find the following works useful:

Gleadow, R. *The Origin of the Zodiac* (Castle, Pasadena, 1968).

Dean, G. *Recent Advances in Natal Astrology* (Analogic, 1977).

Rudhyar, D. *The Astrology of Personality* (Doubleday, New York, 1970).

Meyer, M. *A Handbook for the Humanistic Astrologer* (Anchor, Manhattan Beach, California, 1974).

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(female) test group members to male (female) control members. Thus, the test-control group assignments had to be re-established.

Obviously, since the natal chart depends entirely on the natal data, inaccuracies in the latter would produce inaccuracies in the former. The astrologers insisted that the birth time be accurate to within 15 minutes. In order to assure this, when the subjects took the CPI, they were obliged to show documentation of their natal data including, especially, birth time. Although we preferred birth certificates, hospital and county record or other 'official' documentation, we also accepted baby books provided that the birth time was recorded when the child was born. (A more complete discussion of biases may be found in L.B.L. Preprint No. 20480.)

Double-blind procedures

An important difference between this and many previous tests is our extensive use of double-blind techniques. The important procedures we followed are outlined below.

As soon as subjects had taken the CPI, their questionnaires were put into alphabetical order and given to an assistant, who assigned a five random-digit code number to each in turn. No two subjects were assigned the same code number. The assistant then filled out three 3 × 5 index cards for each subject. On the first he put

Name-Code Number; these he filed in alphabetical order. On the second he put Code Number-Name; these were filed in numerical order. The purpose for these cards was for easy record-keeping. The cards were maintained under the supervision of Prof. Muller, and could be released to the experimenter only with his consent. At no time during the data collection did the experimenter have access to any information relating subjects' identities to code numbers. This control was abandoned only when all the data had been collected and the methods of analysis had been established.

The assistant also made a third set of cards each containing the code number of a particular subject and his natal data. These cards were given to astrologers Michael Caveney and Chris Nelson in envelopes unopened by the experimenter, for the construction of natal charts. Since the CPI was given at three different sessions (typically about 4 weeks apart), not all the assignments were made at the same time, nor did the astrologers receive all the natal data cards at one time. They did receive, however, the natal data cards within five days of the CPI testing dates.

Subjects who failed to show documentation of birth time, date and location when they took the CPI were automatically put into the control group (there were 43 such subjects). All questionnaires in each group were sorted into twelve Sun-sign groups. We then randomly assigned subjects to the control group until the number of control group members equalled the number of subjects remaining in each Sun-sign group. The remaining subjects comprised the test group. If there was an odd number of subjects in a Sun-sign group, the odd person was put in the control group.

The questionnaires in each Sun-sign group were then shuffled thoroughly and the names and birthdates were listed in the order in which they landed. As stated earlier, we required the birth dates to be at least three years apart to ensure that the natal charts were sufficiently dissimilar. The first test subject of each Sun-sign group was then matched to the first control subject of the same group whose birth date was far enough apart. The procedure was repeated for the second, third, etc. test subjects until we were left with test and control subjects whose birthdates were within three years of each other (which happened in all 12 Sun-sign groups).

To include these remaining subjects, we were forced to do some rematching in the

Table 1 Data from subject selections of natal chart interpretations

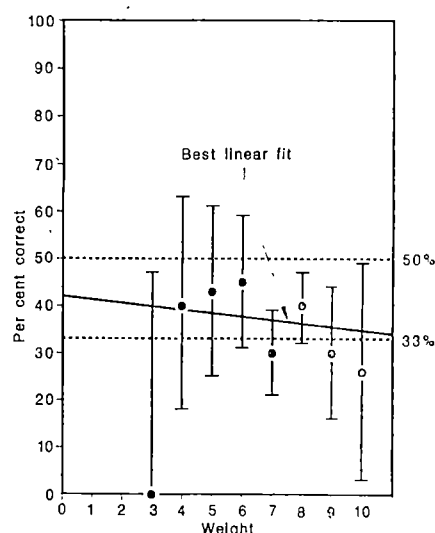
	Total	First choice	Second choice	Third choice		
Test group	56	25	16	15	18.67 ± 3.53	CPI PROFILE
Control group	50	21	13	16	16.67 ± 3.33	SELECTION
Test group	83	28	33	22	27.67 ± 4.29	INTERPRETATION
Control group	94	42	34	18	31.33 ± 4.57	SELECTION

following way. We started at the top of the control group and went down until we found a birthdate which satisfied the requirements that it was at least three years away from the unmatched test person and that the birthdate test group member to which it was attached was at least 3 years away from one of the unmatched controls. We then rematched the originally-matched control group member with the previously unmatched test subject, and matched the unmatched control group member with the previously matched test member. This was continued until all the test and control group subjects were matched. Because seven Sun-sign groups had an odd number of subjects, there remained seven unmatched control group members.

For the second part of the experiment, male (female) test members had to be assigned to male (female) control members. Thus, the above test subject to control subject matching had to be redone. Since the test and control groups were randomly chosen, no changes in them were made. However, all the matchings changed as male (female) test subjects were randomly matched to male (female) control subjects.

During the conduct of the experiment, each subject was given two envelopes, one containing the materials needed for the selection of the natal chart interpretation and the other containing the materials for the selection of the CPI profile. The natal interpretation envelope contained: (1) three natal interpretations; (2) a pretyped sheet on which the subjects were to detail their choices; (3) a questionnaire asking their opinion of astrology and whether they had had their chart done before (See Experiment Design); and (4) a letter explaining how they were to go about making the selections. The CPI envelope contained: (1) three CPI profiles; (2) a preformatted sheet on which the subjects were to detail their first and second place choices, plus 1-10 ratings; (3) a summary of what the CPI scores mean; and (4) a letter explaining how they were to go about making their selections. Since we had labelled the natal charts with the code number of the person for whom they were constructed, we could not do the same with the CPIs, since the subjects might recognize a code number appearing twice. The CPIs were first labelled with the code numbers of the persons to which they corresponded, and then relabelled by finding

Fig. 4 Graph showing percentage correct versus rating for astrologers first place choices in CPI profile natal chart matching. The best linear fit is consistent with the scientifically predicted line of zero slope. No significant tendency for the astrologers to be more correct when they rate a CPI as highly matching a natal chart.



$\ln(1/x)$ for each code number, x , on a hand calculator and taking the last 5 digits on the calculator display as the new code number. All natal interpretations and CPI profiles were put in numerical order in the envelopes.

The astrologers were sent materials in two separate mailings. In the first, each received: (1) the number of natal charts he had agreed to interpret when he chose to take part (typically 4); (2) a copy of the format by which the charts were to be interpreted; (3) the paper with headings on which the interpretations were to be typed; (4) a letter explaining the symbols used on the computer-constructed natal charts, deadlines and notice of when they might expect the materials for part two of the experiment; (5) a postage-paid return envelope. After they had returned the natal charts and interpretations, the astrologers received the second mailing containing: (1) the number of natal charts (plus three CPIs for each natal chart) which they had agreed to match to CPI profiles; (2) a copy of *The Interpreter's Syllabus for the CPI*² (a booklet explaining all the CPI attributes and how to interpret them in detail); (3) a preformatted sheet on which the astrologers were to detail their first and second choices and ratings; (4) a letter explaining how to go about making the choices; (5) a postage-paid return envelope for the data.

To save the astrologers work, they were allowed to make the CPI matchings to the natal charts they had already interpreted. They were also typically sent an additional natal chart and CPIs to match. To ensure that the astrologers would not mix up the CPI profiles between natal charts, the three profiles for each natal chart were ordered randomly, then labelled *a*, *b* and *c* respectively.

A total of 226 natal charts were sent out to be matched with CPI profiles. Of these, only 15 had no documentation of birth time. None of these 15 were returned by the astrologers to be included in our data.

Results

Part 1. Subject selection of natal chart interpretations. After a predetermined data collection period of 10 weeks, we had astrological data from 83 test and 94 control subjects and CPI self-selection data from 56 test and 50 control subjects. The data are displayed in Table 1.

The test group selected the correct interpretation as its first choice at the rate of 0.337 ± 0.052 , the control group at the 0.447 ± 0.049 rate, 2.34 standard deviations above chance. Although this fluctuation is less than 2.5 standard deviations, the level we had chosen to call 'significant', it does require comment. Since this fluctuation occurred in the control group and control subjects were not given their own interpretations, this cannot be interpreted as a possible astrological effect. Neither can it be correctly attributed to Sun/sign bias, since the test group did not score near the same level. We thus interpret this as a statistical fluctuation. The test group chose the correct interpretation as second best, describing them at the 0.398 ± 0.052 rate while the control group did so at the 0.362 ± 0.049 rate. Finally, the correct interpretation fell as the test group subjects third choice at the 0.265 ± 0.052 level and, for the control group, at the $0.191 \pm$

Table 2 Data from astrologers matching natal charts to CPI profiles

	Total (n)	Chance (n/3) [Expected s.d.]	Astrologers predicted (n/2) [Expected s.d.]	No. of correct CPI chosen	Standard deviation away from 0.35	Standard deviation away from 0.50
First choice	116	38.5 [5.1]	58.5 5.4	40	+2.56	3.34
Second choice	114	38.0 [5.0]	None	46	+1.48	—
Third choice	114	38.0 [5.0]	None	28	2.0	—

The data are consistent with chance, inconsistent with astrological hypothesis.

0.049 rate. All this is consistent with the scientific hypothesis.

When the first few data envelopes were opened, we noticed that on any interpretation selected as a subject's first choice, nearly all the subsections were also rated as first choice. We then realized that we had no way of guaranteeing that subjects were rating each section of the interpretations independently of others they had already read. Without such a guarantee, spurious results favouring either hypothesis could have easily appeared. So we rejected these data as not having been collected under the proper controls.

Next we looked to see how well the subjects were able to select the correct CPIs. The test group selected the correct CPI as their first choice at the 0.446 ± 0.063 rate, while the control did so at the 0.420 ± 0.066 rate, showing no significant difference between the two groups. The test group chose the correct CPI as the second place choice at the 0.286 ± 0.063 rate, while the control did so at the 0.260 ± 0.066 rate, again no significant difference between the two groups. Finally, the test group chose the right interpretation as their third choice at the 0.268 ± 0.063 rate while the control group did so at the 0.320 ± 0.066 rate. Once more, there is no significant difference between the two groups.

Part 2. Natal Chart-CPI matching. A total of 116 data envelopes were returned by the astrologers. In the 116 envelopes there was a total of 116 first place choices, 114 second place choices and 320 ranked choices (weight factors indicating how well the astrologers felt the natal chart matched each CPI, on a scale of 1 to 10) (Table 2).

The data were first analysed without taking the 1-10 weight factors into account. The astrologers selected the correct natal chart as their first place choice at the 0.34 ± 0.044 rate, in agreement with the scientific hypothesis of 0.33 and in disagreement with the astrological hypothesis of 0.5 by 3.3 standard deviations. The correct CPI was chosen as the second place choice at the 0.40 ± 0.044 rate which is also consistent with the scientific hypothesis. (The astrologers had made no firm prediction about the second place choice.) The correct CPI was chosen as the third place choice at the 0.25 ± 0.044 rate, again consistent with the scientific hypothesis.

Next we took the weights into account, by a method established before studying the data. (The establishment of methods before data analysis is important in order to prevent the subtle bias that comes from selection of analysis procedures.) We first made a histogram of the weights which the astrologers assigned to all their place choices, regardless of whether or not those choices were correct (Fig. 3). The data are sharply peaked at a weight of about 8. The second histogram in Fig. 3 shows the ratings of only those first place choices which

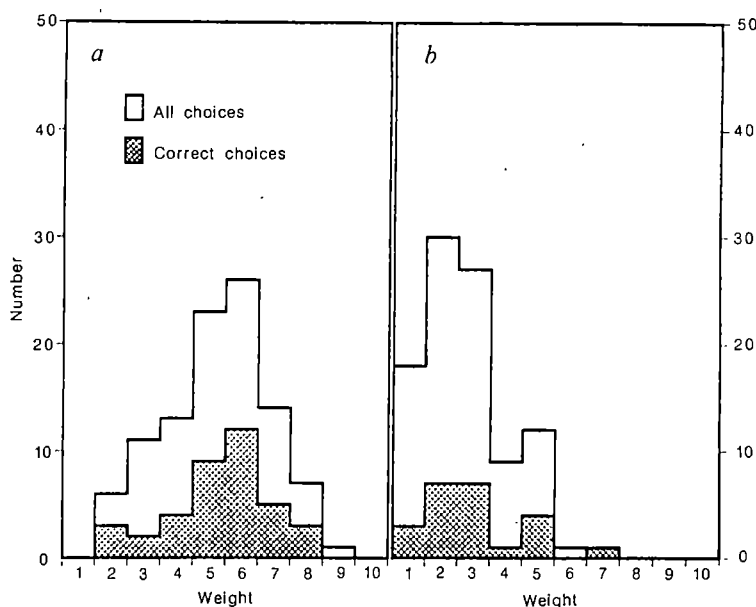


Fig. 5 Histograms showing rates assigned to astrologers' second (a) and third (b) place choices.

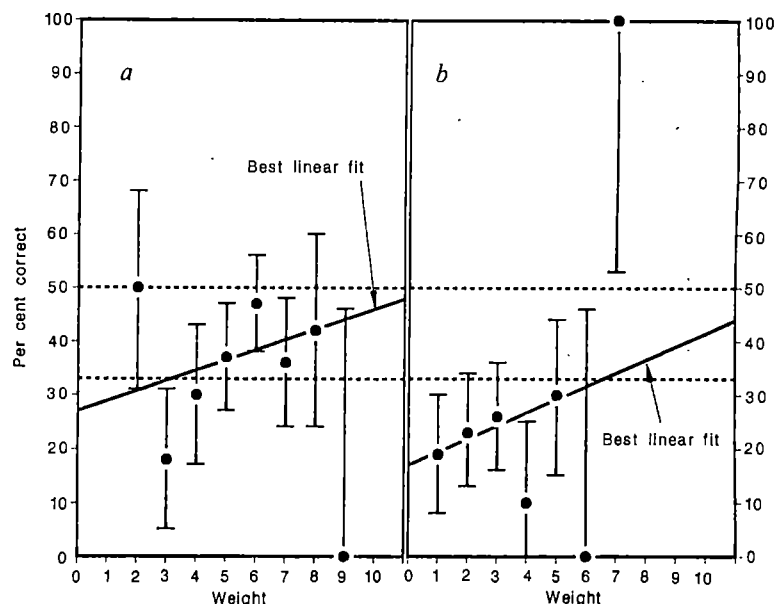


Fig. 6 Percentage of correct CPI profiles versus rates, chosen by astrologers as their second (a) and third (b) place choice. Best linear fits are consistent with chance.

were correct. If the astrological hypothesis were true, one might expect the correct first place choices to have higher weights on average than the whole group of first place choices. Thus, the new histogram should be skewed to the right. On comparing the two histograms, however, we see that they are very similar; no such skewing appears.

The scientific hypothesis predicts that 1/3 of the choices at any weight should be correct choices. Figure 4 shows the percentage correct for each weight with the appropriate error bars, and the best linear fit with slope -0.01 ± 0.02 . The slope is consistent with the scientific prediction of zero slope. The same analysis on the second and third place choices yields Figs 5a, b and 6a, b. The slope of the best linear

fit to the data on Fig. 6a is 0.019 ± 0.02 while that of Fig. 6b is 0.0026 ± 0.02 both consistent with the scientific hypothesis (zero slope).

Conclusions

From the results of Part 1 (subjects selecting interpretations), we notice that the test group scored at a level consistent with chance and within 2.5 standard deviations of the control group. The large (2.34 s.d.) but not significant (less than 2.5 s.d.) fluctuation in the control group is attributable to statistical fluctuation, not to a Sun-sign bias. These results are consistent with the scientific hypothesis. However we cannot use the result to rule against the astrological hypothesis, because the test subjects were also unable to select their

own CPI profile at a better-than-chance level. At the 95 per cent confidence level, the test subjects were unable to select their own CPI profile at better than the 0.57 rate. There are many reasons which could explain why the test subjects were unable to select the correct CPIs at a higher rate:

- (1) Subjects may have had difficulty relating to the graphical presentation of the CPI information.
- (2) Some subjects may have recognized correct information about themselves, but subconsciously chose a CPI which did not describe them as well to avoid admitting they have certain character traits. Such denial in a large percentage of the subjects would tend to cancel a positive effect.
- (3) The CPI may not test the kind of attributes by which subjects may easily recognize themselves.
- (4) People may be unable to recognize accurate descriptions of themselves.

Our experiment does not distinguish between these possibilities. Professor H. Gough (author of the test and respected experimental psychologist) is familiar with nearly all published experiments using the CPI. At our request he searched through the literature for any experiment demonstrating the ability of test subjects to recognize accurate descriptions of themselves. To his and our knowledge, no other test of this kind has ever been done. Thus, we believe there exists presently no scientific evidence from which one can conclude that subjects can select accurate descriptions of themselves at a significant rate.

If subjects cannot recognize accurate descriptions of themselves at a significant level then the experiment would show a null result no matter how well astrology worked. On the other hand, any astrological effect demonstrated in this way would require a consistency check. One would have to see if subjects could recognize the kind of information astrologers give them about themselves, which was derived in a manner *known* to be reliable. Thus, until and unless such a self-recognition ability can be shown, we conclude that subject selection of astrologically derived information is a poor test of astrology. (This is a problem in approximately 30 per cent

of all experiments which claim a significant astrological effect.)

The conclusions to be reached from Part 2 (CPI-natal chart matching) of the experiment are somewhat more illuminating. What is striking about these data is how poorly the astrologers performed, when their performance is compared to their predicted rate. It is consistent with chance, and is at the very significant 3.3 s.d. level below the astrologers' prediction. This is well beyond the 2.5 s.d. requirement we established before the beginning of the experiment as sufficient to refute the astrological hypothesis.

Before the data had been analysed, we had decided to test to see if the astrologers could select the correct CPI profile as either their first or second choice at a higher than expected rate. The scientific hypothesis predicts the CPI will fall in the first or second choice 66 per cent of the time. The astrologers did not make a specific prediction as to what they expected the rate to be. If the correct CPIs are chosen in the first and second place choices, then they will be depleted from the third place choice. Since the rate at which the astrologers chose the correct CPI as their third place choice was consistent with chance, we conclude that the astrologers were unable to choose the correct CPI as their first or second choices at a significant level.

In Fig. 4 the data are clearly inconsistent with the 'at least' 0.5 level predicted by the astrologers. Nor do the data suggest that the astrologers are more likely to be correct when they rate a CPI as well fitting the particular natal chart than they are when they weight it as poorly fitting the natal chart. The data appear randomly scattered about the 0.33 line and is hence consistent with chance. The scientific hypothesis predicts a line of zero slope, consistent with the slope observed. Figures 5 and 6 likewise show no convincing evidence that the astrologers tended to rate the correct CPIs higher than the incorrect CPIs.

We are now in a position to argue a surprisingly strong case against natal astrology as practised by reputable astrologers. Great pains were taken to insure that the experiment was unbiased and to make sure that astrology was given

every reasonable chance to succeed. It failed. Despite the fact that we worked with some of the best astrologers in the country, recommended by the advising astrologers for their expertise in astrology and in their ability to use the CPI, despite the fact that every reasonable suggestion made by the advising astrologers was worked into the experiment, despite the fact that the astrologers approved the design and predicted 50 percent as the 'minimum' effect they would expect to see, astrology failed to perform at a level better than chance. Tested using double-blind methods, the astrologers' predictions proved to be wrong. Their predicted connection between the positions of the planets and other astronomical objects at the time of birth and the personalities of test subjects did not exist. The experiment clearly refutes the astrological hypothesis.

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Direct observation of He^+ pick-up ions of interstellar origin in the solar wind

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Singly-ionized helium with a velocity distribution extending up to double the solar wind velocity has been detected in interplanetary space. This distribution unambiguously determines the source: interstellar neutrals, ionized and accelerated in the solar wind. The observed significant flux increase in early December is due to the gravitational focusing of the interstellar neutral wind on the downwind side of the Sun.

THE penetration of the interstellar medium into the heliosphere and the interaction between the solar wind and the interstellar gas have been of great interest for many years¹⁻⁴. The first experimental evidence of neutral interstellar hydrogen penetrating into the heliosphere was obtained from Lyman α sky background mapping^{5,6}. Similar observations of interstellar helium using the He I 584-Å resonance line⁷⁻⁹ revealed the existence of an interstellar neutral wind in interplanetary space which is subjected to the forces of solar gravitation and radiation pressure. When approaching the Sun, the interstellar gas is ionized by solar ultraviolet radiation, by charge exchange with solar wind ions and by collisions with solar wind electrons. The newly created ions are then picked up by the solar wind through interaction with the interplanetary magnetic field.

While most of the constituents are already ionized far beyond the orbit of the Earth, neutral helium (because of its high ionization potential) approaches the Sun to <1 AU (ref. 1). Therefore, a significant fraction of the helium is ionized inside the Earth's orbit and one would expect that these ions are observable by spacecraft in the solar wind. So far, no conclusive measurement on He^+ ions of interstellar origin has been given. Although signatures of He^+ in the solar wind with varying abundances have been reported occasionally^{10,11}, a systematic search for a permanently present flux of interstellar He^+ as part of the solar wind resulted only in upper limits more than one order of magnitude below the estimated flux levels¹². After this negative result Vasyliunas and Siscoe¹³ suggested that the newly created He^+ ions may not be thermalized rapidly enough to occur within the solar wind distribution. The expected broad energy and angular distribution of the interstellar helium ions, however, was not visible to the particle detectors flown on spacecraft up to now. A search for interplanetary He II emission at 304 Å was also not conclusive¹⁴. In this article, we will report measurements with a time-of-flight (TOF) spectrometer in which the interstellar helium ions and their velocity distribution in the solar wind are clearly identified for the first time.

Instrumentation and spacecraft

The data presented here were obtained with the SULEICA (Supra-thermal Energy Ionic Charge Analyser) instrument of the Max-Planck-Institut and the University of Maryland onboard the IRM spacecraft of the AMPTE (Active Magnetospheric Particle Tracer Explorer) project. The IRM was launched on 16 August 1984, into a highly elliptical orbit with an apogee of $18.6 R_E$. During the time period from launch until December 1984 the satellite spent a large fraction of each orbit in the solar wind upstream of the Earth's bow shock.

The SULEICA instrument combines the selection of incoming ions according to their energy per charge by electrostatic deflection with a subsequent TOF analysis and the final measurement of the residual ion energy in a silicon surface barrier detector. The energy range from 5 to 270 keV/charge is covered by stepping the analyser voltage in 24 logarithmically spaced voltage steps in synchronism with the spacecraft spin. The fan-like

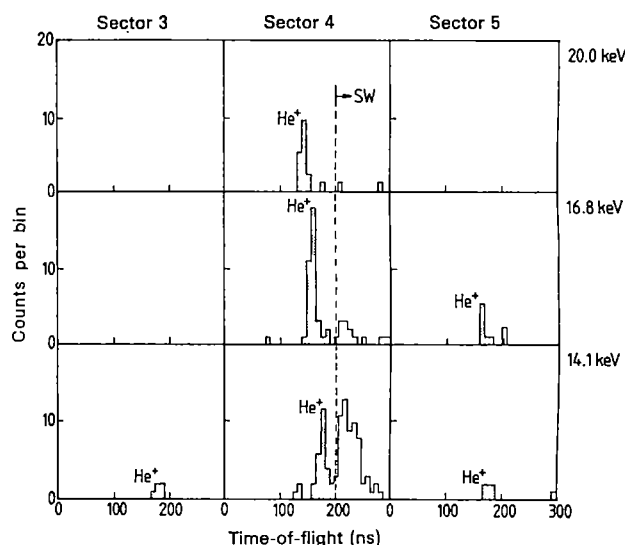


Fig. 1 Typical TOF-histograms at three different energy steps taken in the Sun sector and the two adjacent sectors. The data were obtained during a period of 45 min on 11 November 1984, at $\sim 18 R_E$ in front of the Earth's bow shock.

aperture of the electrostatic analyser (two concentric segments of a sphere) covers a solid angle of 10° in azimuth and 40° in elevation symmetric to the plane perpendicular to the spacecraft spin axis. The directional information in azimuth is provided by a sectoring scheme with 16 sectors for protons and α particles and eight sectors for the other ions. The geometrical factor of the instrument is $4.3 \times 10^{-2} \text{ cm}^2 \text{ sr}$ and its energy resolution is $\Delta E/E \approx 0.097$. A more detailed description may be found elsewhere¹⁵.

The expected energy of the pick-up ions (≤ 40 keV) is too low to create energy signals significantly above the noise level of the solid state detectors. Therefore, the ion species are identified by combining the electrostatic deflection (E/Q) and the TOF signal. For each specific E/Q step, the TOF distribution represents a unique mass-per-charge distribution, since $M/Q \sim E/Q \times \text{TOF}^2$. Recently this type of analysis has been successfully applied to identify Li^+ pick-up ions after the lithium vapour cloud releases of the AMPTE project in the solar wind¹⁶.

Basic observations

For the present investigation a limited number (12) of observational periods in the solar wind were chosen between launch of the spacecraft and the end of December 1984. During all periods we observe a significant peak at $M/Q = 4$ in the TOF-histograms for E/Q steps which correspond to energies higher than we would expect for genuine singly charged solar wind helium. Depending on the E/Q step we also observe a broad and variable peak at TOF-values which correspond to the actual

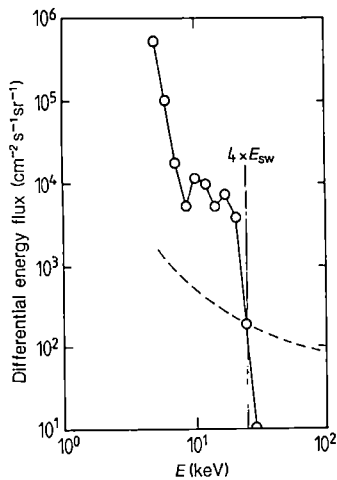


Fig. 2 Differential energy flux spectrum of the $M/Q=4$ channel taken in the Sun sector with an accumulation time of 40 min. The dashed line represents the 1 count level for each energy.

solar wind velocity. These ions with M/Q values well above 4 represent heavy ions of the solar wind. In addition, the signature of the He^+ ions at $M/Q=4$ is also seen in the two sectors adjacent to the sunward direction. A typical example of TOF-histograms for three different voltage steps and all three azimuthal sectors with the He^+ peak is shown in Fig. 1. The sample was taken during a 45-min interval on 11 November 1984, when no energetic ions occurred upstream of the bow shock. Note, however, that the He^+ signature is visible at all orientations of the interplanetary magnetic field and is independent of whether or not upstream particles are present.

Figure 2 shows the differential energy flux spectrum of the He^+ ions in the Sun sector for the same time period. The spectrum is basically flat between ≈ 8 and 22 keV with a sharp cutoff at ≈ 22.3 keV. This value corresponds to about four times the solar wind bulk energy of helium (indicated by the vertical line) for this time period. The actual solar wind velocity was 550 km s^{-1} , as derived from the data of the Max-Planck-Institut/University of California Berkeley three-dimensional-Plasma instrument on IRM¹⁷. Below 8 keV a sharp rise of the spectrum occurs which is attributed to solar wind ions with $M/Q=4$, for example, Si^{7+} and S^{8+} (ref. 12). The dashed line in Fig. 2 indicates the one count limit for He^+ .

Signature of pick-up ions

In contrast to genuine solar wind ions heavier than protons, which flow with almost solar wind bulk velocity, freshly created ions in interplanetary space are initially at rest. After ionization they are immediately subjected to the combined forces of the interplanetary $v_{\text{sw}} \times B$ electric field and the magnetic field B where v_{sw} is the solar wind velocity. In the inertial system (which coincides with the rest frame of the spacecraft), the ions initially perform a cycloidal motion perpendicular to the local magnetic field. The velocity varies between basically zero (the velocity of the neutral wind at $\approx 20 \text{ km s}^{-1}$ is neglected compared with the solar wind velocity) and a maximum value

$$v_{\perp \text{max}} = 2 v_{\text{sw}} \sin \alpha \quad (1)$$

which is determined by the solar wind velocity and the angle α between its flow direction and the local magnetic field. The maximum energy of pick-up ions then is

$$E_{\perp \text{max}} = 4(M/2)v_{\text{sw}}^2 \sin^2 \alpha \quad (2)$$

In the solar wind frame, the pitch angle distribution of these particles is a ring in velocity space with the pitch angle α , that is, the ions gyrate with $v_{\perp} = v_{\text{sw}} \sin \alpha$ and move along the magnetic field with $v_{\parallel} = v_{\text{sw}} \cos \alpha$. Observations of artificially-injected lithium in the solar wind which have been made within the first cycloid after the ionization are compatible with such

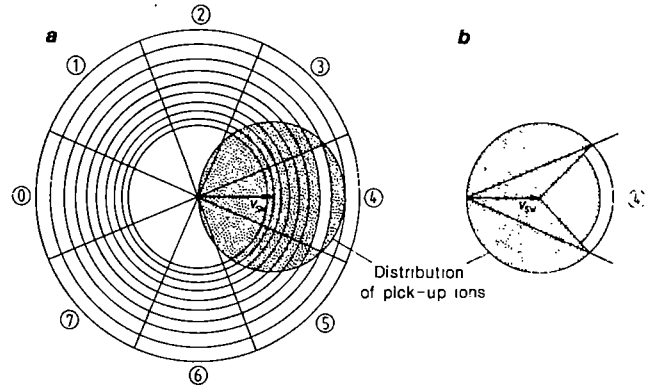


Fig. 3 a, Cut through the fully developed velocity distribution of pick-up ions in the spin plane of the spacecraft (shaded area) along with the sectoring and voltage stepping scheme of the instrument. The velocity space element, which is used to determine the differential energy flux as seen by the instrument, is left blank. b, Similar representation in the solar wind frame. The velocity space element, over which the actual integration is performed, is left blank.

an undisturbed pick-up distribution¹⁶. Our observations of He^+ ions, which are spread over a large volume in velocity space, seem to indicate a significant broadening of the initial ring distribution in pitch angle as well as in energy.

Cosmic rays are strongly pitch-angle-scattered by magnetic field fluctuations in the solar wind, while their energy remains almost unaffected¹⁸. The corresponding mean free path for scattering of He^+ pick-up ions (with a typical magnetic rigidity of 5–10 MV) turns out to be about 0.05 AU according to a compilation of cosmic-ray propagation parameters¹⁹. From these arguments, pitch angle scattering seems to be rather efficient for pick-up ions, and shortly after ionization a spherical shell distribution in velocity space is formed within the rest frame of the solar wind. Initially, the ions are injected into the solar wind with the energy $E_0 = (M/2)v_{\text{sw}}^2$ (with velocity components v_{\parallel} and v_{\perp} as discussed above). Therefore, the radius of the spherical shell distribution is equal to the solar wind velocity. Subsequently, the ion energy decreases as a result of adiabatic deceleration during the radial transport with the expanding solar wind. Due to adiabatic deceleration the energy of fully pitch-angle-scattered particles varies like

$$E/E_0 = \left(\frac{r_i}{r}\right)^{4/3} \quad (3)$$

with the distance r from the Sun, starting at the location of birth of the ion r_i (ref. 13). This relation immediately leads to a mapping of the spatial source distribution of pick-up ions upstream of the spacecraft into an energy or velocity distribution in the solar wind frame at the location of the satellite which can be written as:

$$f(v) = \frac{3N_0 v_{\text{ion}} r_0}{8\pi v_{\text{sw}}^4} \left(\frac{v}{v_{\text{sw}}}\right)^{-3/2} \quad (4)$$

N_0 and v_{ion} are the neutral density and the ionization rate of the interstellar gas at the Earth's orbit ($r_0 = 1 \text{ AU}$). v_{ion} is assumed to vary like $1/r^2$ with the intensity of solar ultraviolet radiation. In the spacecraft frame of reference, this results in a particle distribution which extends between $E=0$ and a maximum energy

$$E_{\text{max}} = 4E_{\text{sw}} = 4(M/2)v_{\text{sw}}^2 \quad (5)$$

for particles coming from the sunward direction. Such a distribution with a more or less flat spectrum in differential energy flux is, indeed, observed between about 8 and 22 keV (see Fig. 2).

From these observations, it can be concluded that the velocity distribution of the pick-up ions is basically a full sphere centred at the solar wind velocity. A cut through this distribution in the plane perpendicular to the spin axis of the satellite is shown in Fig. 3. The azimuthal sectoring scheme and the voltage stepping

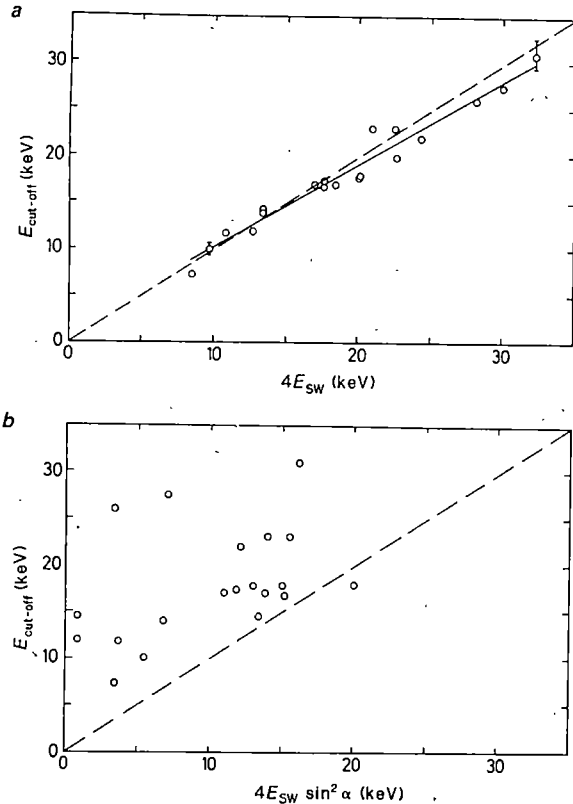


Fig. 4 Correlation of the experimental cutoff energies of the He^+ spectrum with the fourfold solar wind bulk energy $4E_{\text{SW}}$ of helium (a) and with the maximum pick-up energy perpendicular to the interplanetary magnetic field $4E_{\text{SW}} \sin^2 \alpha$ (b).

scheme (concentric circles) are overlaid. This interpretation is also supported by the TOF histograms shown in Fig. 1, where the He^+ peak is observed in the Sun sector 4 as well as in the neighbouring sectors 3 and 5. The energy spectrum displayed in Fig. 2 represents a cut through the distribution in the Sun sector. It follows from the measured flat energy spectrum that a large fraction of the sphere in velocity space rather than only the outer shell is filled with He^+ ions. Otherwise, a significant flux decrease between E_{SW} and $4E_{\text{SW}}$ would be expected. Therefore, a significant part of the He^+ population originates from locations several tenths of an AU upstream of the spacecraft. The energy of these ions has decreased on their way according to relation (3).

The energy spectra of the He^+ ions have been investigated for several time periods of different solar wind velocities and interplanetary magnetic field directions. In Fig. 4, the observed cutoff energies E_{cutoff} (defined by a decrease of the differential energy flux by a factor of 10 compared with the mean plateau level) are plotted first against the fourfold solar wind bulk energy $4E_{\text{SW}}$ of helium as given by relation (5) and second against the initial maximum pick-up energy $4E_{\text{SW}} \sin^2 \alpha$ according to relation (2). There is an excellent correlation with the full four-fold solar wind energy (correlation coefficient 0.98), while the correlation with the maximum pick-up energy is very poor (correlation coefficient 0.36). This result clearly indicates that the pick-up ions in all conditions have mainly lost their directional information due to pitch angle scattering and that most of the ions originate from regions beyond one mean free scattering length (0.05 AU) upstream of the observer.

Note that the generation of several types of plasma waves by the highly unstable distribution of pick-up ions has been discussed in the past^{20,21}. These waves were expected to lead to a complete thermalization in energy of the pick-up ion distribution in the solar wind frame of reference in addition to pitch angle scattering. However, the results described above—in particular, the sharp cutoff in energy—indicate that the influence of these waves on the He^+ distribution can be neglected compared with the adiabatic cooling.

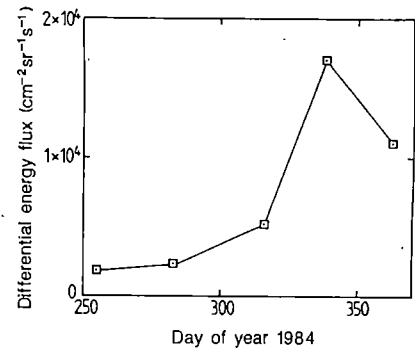


Fig. 5 Differential energy flux of 20-keV He^+ ions obtained in the Sun sector for five different time periods between September and December 1984.

Source of the He^+ ions

Knowing the velocity distribution of the He^+ ions as shown in Fig. 3, the source strength $S = N_0 v_{\text{ion}}$ of the pick-up ions can be obtained from the measured differential energy flux at a fixed energy. The differential energy flux can be calculated by integrating the distribution function (relation (4)) over one voltage step and the Sun sector in the spacecraft frame (blank area in Fig. 3a). Instead of performing the full transformation from the solar wind frame into the spacecraft frame we use the fact that the distribution is isotropic in the solar wind frame and that near the maximum energy of the ion distribution (in the Sun sector) the volume element in velocity space $\Delta \Omega v^2 \Delta v$ covered by one energy channel of the instrument appears to be extended by a factor of four in solid angle in the solar wind frame compared with the spacecraft frame as indicated in Fig. 3b. Within this approximation, the integration is performed over the corresponding sector in the solar wind frame (blank area in Fig. 3b). Using the distribution function (5) we obtain for the differential energy flux:

$$\frac{\Delta J E}{\Delta E} = \frac{3 N_0 v_{\text{ion}} r_0 E}{8 \pi \Delta \Omega \Delta E} 4 \Delta \Omega \frac{1}{v_{\text{sw}}^4} \int_{0.9 v_{\text{sw}}}^{v_{\text{sw}}} \left(\frac{v}{v_{\text{sw}}} \right)^{-3/2} v^3 dv \quad (6)$$

According to the energy resolution of the instrument ($\Delta E/E \approx 0.1$, corresponding to $\Delta v/v \approx 0.05$) the integration limits in velocity are $0.9 v_{\text{sw}}$ and v_{sw} in the solar wind frame. Note that the differential energy flux (compared with the differential particle flux $\Delta J/\Delta E$), when taken at the maximum energy of the pick-up distribution, does not vary with the solar wind velocity. Therefore, this quantity is a suitable parameter for subsequent long-time studies of the He^+ source. Using $w = v/v_{\text{sw}}$ we find:

$$\frac{\Delta J E}{\Delta E} = \frac{3}{2\pi} N_0 v_{\text{ion}} r_0 \frac{E}{\Delta E} \int_{0.9}^1 w^{3/2} dw \approx 0.5 N_0 v_{\text{ion}} r_0 \quad (7)$$

Taking an ionization rate $v_{\text{ion}} = 1.25 \times 10^{-7} \text{ s}^{-1}$ at 1 AU (ref. 9) a value of $N_0 r_0 = 1.2 \times 10^{11} \text{ cm}^{-2}$ is derived from the mean differential energy flux between 16 and 20 keV ($7 \times 10^3 \text{ s}^{-1} \text{ cm}^{-2} \text{ sr}^{-1}$) on 11 November 1984. The actual column density upstream of the spacecraft corresponds to about half the value of $N_0 r_0$.

Possible sources for singly charged ions which are injected into the solar wind with zero velocity are the interstellar neutral gas as discussed above and, in addition, planetary atmospheres and cometary comae. Of the latter ones the only source of interest for the observations reported here is the Earth's atmosphere. In contrast to the interstellar gas, which is present over a large scalelength in interplanetary space, the terrestrial sources of neutral helium are rather localized. Because the density of the exosphere typically varies like $1/R^2$ with distance from the Earth, the remaining scalelength of Earth's exosphere (the outer regime of the atmosphere) beyond the location of the spacecraft ($18 R_E$) is of the order of $20 R_E$. Therefore, a terrestrial origin can be ruled out for the observed He^+ ions for two reasons.

First, the observed velocity distribution can only be explained in terms of a source which extends far beyond the mean free

scattering length of ~ 0.05 AU upstream from the Earth, while the terrestrial exosphere has a scalelength of typically $20 R_E$.

Second, with a scalelength of $\sim 20 R_E$ the measured column density would lead to a helium density of $\sim 5 \text{ cm}^{-3}$ at a distance of $18 R_E$. This value exceeds densities known for exospheric helium by more than 3 orders of magnitude²².

Therefore, the observed He^+ ions are most likely of interstellar origin. The corresponding helium density in interplanetary space upstream of the spacecraft on 11 November was determined to be $N_0 \approx 8 \times 10^{-3} \text{ cm}^{-3}$.

The flux of He^+ ions and, therefore, the neutral density of helium shows a significant variation over the observation period from September to December 1984. In Fig. 5, the differential energy flux of He^+ at 20 keV is plotted against time. For this analysis, only those time periods were taken when the solar wind velocity exceeded 550 km s^{-1} ; that is, the maximum energy E_{cutoff} was $> 20 \text{ keV}$. The differential energy flux is low in September and October ($\sim 2 \times 10^3 \text{ s}^{-1} \text{ cm}^{-2} \text{ sr}^{-1}$). An increase by about a factor of 10 is observed until early December, after which the flux falls off again towards the end of December. This result is in good agreement with models of the spatial distribution of neutral helium in interplanetary space and the observations of $\text{He I } 584\text{-}\text{\AA}$ radiation^{8,9}. During December, the Earth is on the downwind side of the interstellar neutral wind with respect to the Sun. Here the helium is supposedly focused by the gravitational forces of the Sun and, therefore, the local density is enhanced significantly. The observation of this effect constitutes additional evidence for the interstellar source of the He^+ ions.

These observations are of particular importance, since various parameters of the local interstellar medium can be derived from the annual variation of the flux of He^+ ions: (1) the location of the apex; (2) the value of the relative velocity between the sun and the local interstellar medium; (3) the temperature; and (4) the density of the interstellar helium.

The absolute value of the density near the Earth as given above is compatible with results reported from ultraviolet measurements^{8,9}. A quantitative analysis of the density outside the heliosphere and the determination of the parameters of the local interstellar medium as listed under items (1) to (4) above requires a detailed comparison of the experimental results with

theoretical models of the neutral helium distribution in the heliosphere. This is beyond the scope of the present paper and will be presented in future work.

Furthermore, these pick-up ions represent a source of ions with a velocity distribution clearly distinguishable from the solar wind. To study their subsequent acceleration at the Earth's bow shock and at interplanetary shocks will be of great interest. Finally, these ions are most likely the seed particles for the so-called anomalous component of cosmic rays for which an interstellar origin and subsequent acceleration in the interplanetary medium²⁴ or at the terminating shock of the heliosphere²⁵ has been proposed.

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Axial processes along a segment of the East Pacific Rise, 10° – 12° N

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Axial segments of the East Pacific Rise are made up of individual volcanoes. Each volcano has a distinct magma composition and shows a systematic variation in the fractional crystallization with distance along the axis from the central chamber. Hydrothermal venting, lava morphology and tectonics also vary along the axis. Lavas erupted near transform faults, from the tips of propagating rifts in overlapping spreading centres, and from near-axis seamounts have different and variable compositions.

MID-OCEAN ridge spreading centres have recently been shown to consist of discrete segments separated by transform faults and conjugate spreading centres (also called overlapping spreading centres (OSC) or non-transform offsets^{1,2}). Typically, in each segment the axial depth shows a minimum or topographic high at some point between the two ends^{1–4}. Francheteau and Ballard⁴ have proposed that the topographic highs lie above the principal magma chambers feeding lavas to the axis and are thermal expressions of such chambers. Their model predicts that relative volumes of lava and morphology of lava flows

should change systematically along the axis away from the topographic high. Specifically, pillow lavas should increase relative to sheet flows, fracturing should increase and hydrothermal activity should decrease along-ridge away from the topographic high. To date, observations supporting this hypothesis have been made at several locations of different spreading rates, but have been limited to a few kilometres along the axis in the vicinity of the axial topographic high.

We report here on our recent observations over a 180-km length of East Pacific Rise (EPR) axis between 10° and 12° N,

made specifically to test the Francheteau–Ballard model. This location (see Fig. 1) was deliberately chosen because the region had been recently mapped using SEABEAM⁵ and the axial depth and segmentation of the ridge was known. Starting at a major transform fault, the Clipperton Fracture Zone, the neovolcanic zone or zero-age axial depth shows a progressive shallowing to a first topographic high about 10°55' N, deepens to a topographic low, thought to be a small OSC at 11°15' N (ref. 1), rises to a second topographic high at 11°30' N, then deepens to a large OSC at 11°45' N with a distinct basin separating the overlapping ridge segments (Fig. 1). Previously, a French submersible dive^{6–8} on the second topographic high at 11°30' N had indicated intense (black smoker) hydrothermal activity. Thus, all the ingredients were present to test how axial processes varied along the axis with respect to major features such as topographic highs, OSCs and transform faults, and how (or if) magmatic activity and composition varied with distance from the proposed central magma chamber(s).

Variation along axis

Near the Clipperton Fracture Zone at the southern end of the studied ridge segment, the axial graben is not well developed, abundant fissures and talus indicate dominance of tectonic activity, and pillow lavas are the major eruptive form (but are often badly cracked; see Fig. 2a). These observations indicate that eruptions are infrequent. No hydrothermal activity was observed. Halfway along the segment between the Clipperton Fracture Zone and the first topographic high, the axial graben becomes well defined, although fissures are abundant near the graben walls. Within the graben, glassy lobate flows and broken platy sheet-flow lavas (Fig. 2b) indicate relatively recent eruptions, although tectonic activity is still noticeable. Some non-active hydrothermal formations were observed, although these are rare (Fig. 2c). At the first topographic high at 10°55' N (see Fig. 1b), the axial graben is very well developed, undeformed sheet-flow lavas (Fig. 2d) dominate in the graben floor, and hydrothermal activity, including white and black 'smokers', is common (Fig. 2e). Fissuring and faulting near the graben edges are not as abundant as farther south in the segment. Constructive volcanism dominates the topography. Glassy reflective flows are not common in the photographs but sampling recovered very fresh, glassy fragments of sheet flows and lobate flows with a thin mantling of iron-rich hydrothermal precipitate that dulls the surfaces.

The topographic low between the two highs, although previously indicated¹ to be an OSC, does not appear to have typical OSC features. The axial graben appears to be continuous and fresh lava flows and hydrothermal activity are seen. We infer, based on the observations and the magma chemistry, that the axial portion from the northernmost topographic high at 11°30' N has propagated southwards and linked up with the southern axis. The second topographic high at 11°30' N (see Fig. 1b) also shows a well-developed axial graben, sheet flows dominate, and hydrothermal activity with concomitant vent communities and biota are abundant. A cross-section across the graben here, as at the first topographic high, shows only pillow lavas outside the graben, fissuring near the graben wall, talus, collapse pits and flow-back structures in the graben close to the wall, and sheet flows and lava 'ponds' flooring the graben. Hydrothermal activity is found only within or at the walls of the graben⁹. These observations indicate that the sequence of eruptions in the graben must be sheet flows, followed by lobate flows, and finally by pillow lavas as the graben fills. Eventually, the ridge splits, a new graben is formed, and the cycle repeats.

The axial region north of the second topographic high at 11°30' N shows a marked transition as it becomes part of the OSC. Tectonically undisturbed lava formations are less abundant, pillow lavas and talus dominate, the axial graben shallows or pinches out, and hydrothermal activity is not seen. This contrasts markedly with the eastern limb of this OSC which shows active hydrothermal vents, recent sheet flows, and a well developed graben. We believe this limb is active and is propagat-

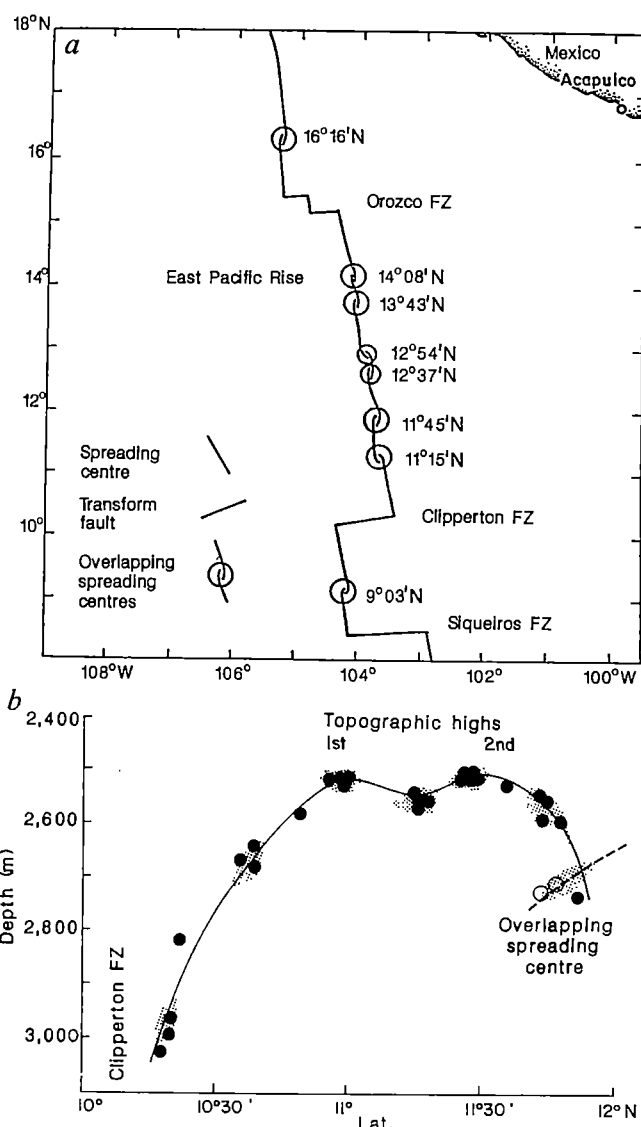


Fig. 1 a, EPR axis showing location of transform faults and conjugate (overlapping) spreading centres. The region studied in this work is the EPR axis from the Clipperton Fracture Zone to, and including, the OSC at 12° N. b, Axial depth versus latitude for the EPR 10°–12° N. The hatched areas are regions photographed using transponder navigation. ●, Dredge locations; ○, dredge locations in the east limb of the OSC. We placed transponders at seven discrete localities along the 180 km of EPR axis and performed detailed ANGUS¹⁴ photography over 4–7 km of the central axial region (see Fig. 1b for location of nets). This photographic coverage allowed us to delineate the axial graben and document such features as lava morphology, relative ages of eruptions, tectonic activity and hydrothermal activity. Using the transponder nets and information from the bottom photographs, we did carefully navigated dredging within the nets and within the neovolcanic zone, plus some dredging between nets using the SEABEAM bathymetry and conventional 12-kHz echo-sounding to guide the sampling (see Fig. 1b for sample location). In all, we made 14 ANGUS runs and 23 successful dredge hauls. About 70 samples initially selected as representative of type from each locality were analysed for major and trace elements by X-ray fluorescence spectroscopy at Woods Hole¹⁵, glass margins were analysed using an electron microprobe at the Smithsonian Institution¹⁶, and petrographic studies and mineral analyses were made on selected thin sections. These preliminary data are presented here.

ing southwards. The nodal basin between the overlapping limbs appears to be a passive feature. The walls are composed only of lava spillage or talus from the respective axes and the floor is covered with sediment, with only a few, old pillow lavas showing through the sediment (Fig. 2f). There appears to be no current volcanic activity within the OSC basin, and no tectonic activity or faulting suggestive of tensional or transform move-

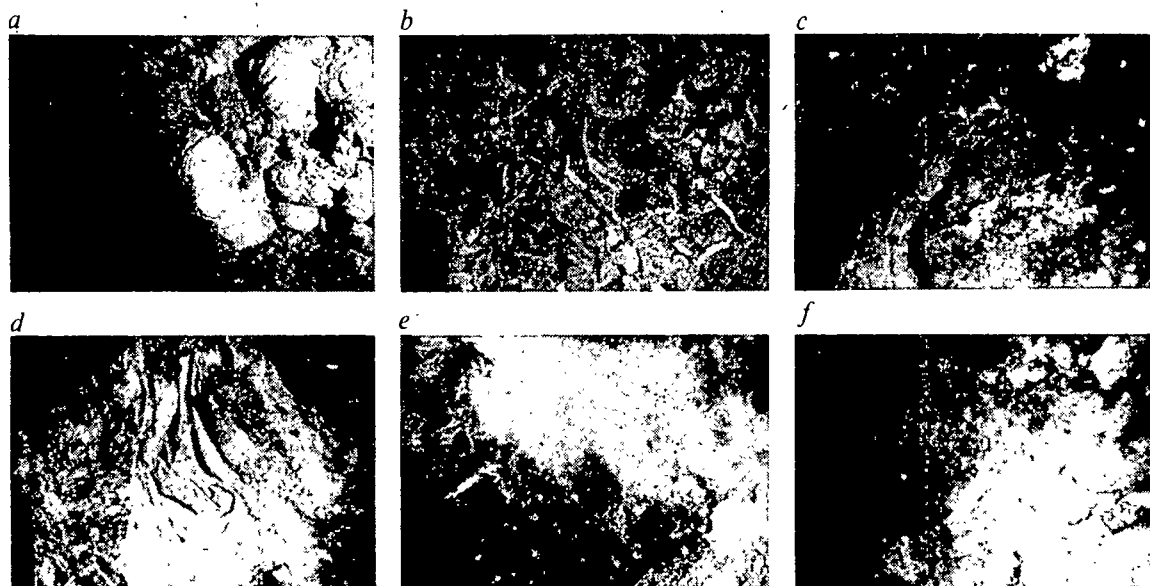


Fig. 2 *a*, EPR axis near the Clipperton Fracture Zone showing typical pillow formations, often cracked. *b*, EPR axis a few kilometres north of *a* location, showing cracked and broken sheet-flow plates in axial graben. *c*, EPR axis about halfway between the Clipperton Fracture Zone and the first topographic high, showing an extinct hydrothermal vent with dissolving clam shells in collapsed lobate flow in the axial graben. *d*, EPR axis at first topographic high showing well developed and fresh sheet-flow formations on floor of axial graben. *e*, Hydrothermal 'smoker' near wall of axial graben on topographic high. Such vents are common on both axial topographic highs. *f*, Floor of nodal basin between the two axial limbs of the OSC at 12° N. The floor is undisturbed and sediment-covered with a few old pillow-lava formations showing.

ment between the limbs as previously suggested for such features¹⁰.

Various petrographic and chemical discriminants allow us to define five magma groups, A-E (Fig. 3). The lavas of magma groups B and C along this segment of the EPR show a systematic variation in Mg number ($\text{Mg}/(\text{Mg} + \text{Fe}) \times 100$) (Fig. 3*a*). With the exception of a few relatively 'primitive' members of magma group A, eruptives farthest from the topographic highs have lowest Mg numbers, suggesting possible cooling and fractionation as magma migrates along-rift. Group B basalts at the low point near the Clipperton Fracture Zone are apparently most fractionated, and there is a systematic decrease in the implied degree of fractionation approaching each topographic high. Basalts increase again in apparent degree of fractionation as the axis deepens into the large northernmost OSC. Both limbs of the OSC, including the tip of the propagating eastern limb, have these apparently fractionated basalts. The systematic compositional variation is reflected in covariances of most chemical components and by the modal mineralogy and mineral compositions. The most fractionated group B varieties, as inferred from Mg number, are moderately phyrlic basalt with up to 25% plagioclase, pyroxene, and minor olivine phenocrysts. Approaching the topographic highs the basalts become less phyrlic, only plagioclase and minor pyroxene or olivine are present, and An and Fo contents increase. Around the first topographic high the basalts are aphyric, and only minor plagioclase microphenocrysts are present. From 11°15' N to the second high, plagioclase plus olivine microphenocrysts are common, while pyroxene is present only near 11°15' N. The most phyrlic group C basalts (~15% plagioclase, olivine and pyroxene) are at the north end of the rift, in the overlap zone at about 11°50' N. Preliminary calculations indicate that on the first topographic high and along the southern limb to the Clipperton Fracture Zone, with the exception of a few basalts nearest the fracture zone, the basalts are comagmatic and simple low-pressure fractionation can be modelled using observed mineral and magma compositions. Similar relations have been demonstrated within the second topographic high and its axial limbs (W.B.B. and K.H., in preparation).

Some of the differences in both major- and trace-element chemistry of the five different magma types can be seen in Fig. 3. A plot of FeO versus MgO (Fig. 3*b*) shows consistent but subtle

differences between groups B and C, while the group of lavas near the Clipperton Fracture Zone (magma type A), and the magmas in the eastern limb of the OSC (magma type D) are clearly defined. Magma group E is found at two locations on the northernmost topographic high; it is characterized by large enrichments in incompatible elements compared with the other basalts from the EPR axis and is similar to transitional or enriched mid-ocean ridge basalts (E-MORB). The most distinctive chemical features of these five magma types are shown in Table 1.

Basalts of magma type A near the Clipperton Fracture Zone occur in the vicinity (same dredge haul) of other basalts belonging to magma type B. However, as noted in the photographic evidence, the axial region is badly broken up and different eruptive units and ages are in close proximity in fault scarps and talus. The basalts of magma type A show a wide range in degree of fractionation compared with the adjacent members of type B magma, which are all relatively fractionated. Compared with magma B, magma A shows lower Fe and higher Ti content for a given MgO concentration. Trace-element contents also are distinctive compared with magma type B, with lower Sr contents (see Fig. 3*c*), lower Zr/Y, and higher Zr/Nb ratios (see Fig. 3*d*). Langmuir and Bender¹¹ have previously noted that basalts erupted close to major transforms have different and unique compositions compared with adjacent axial basalts

Table 1 Chemical characteristics of different magma types along EPR axis 10°–12° N

	Type A	Type B	Type C	Type D	Type E
MgO (wt %)	5.5–7.4	5.6–7.2	6.4–8.1	6.1–6.8	6.6–7.4
FeO* (wt %)	10.9–13.2	10.4–13.8	9.2–11.9	12.2–13.7	9.4–10.0
TiO ₂ (wt %)	1.6–2.4	1.7–2.6	1.5–2.2	2.0–2.4	2.1–2.3
K ₂ O (wt %)	0.14–0.25	0.12–0.25	0.11–0.22	0.09–0.12	0.62–0.73
Sr (p.p.m.)	78–122	129–140	141–168	93–100	196–320
Zr/Nb	39–52	25–35	20–30	38–44	8.7–10.2
Zr/Y	2.4–3.2	2.9–3.0	3.0–3.3	2.5–2.7	4.1–4.6

* Total Fe as FeO.

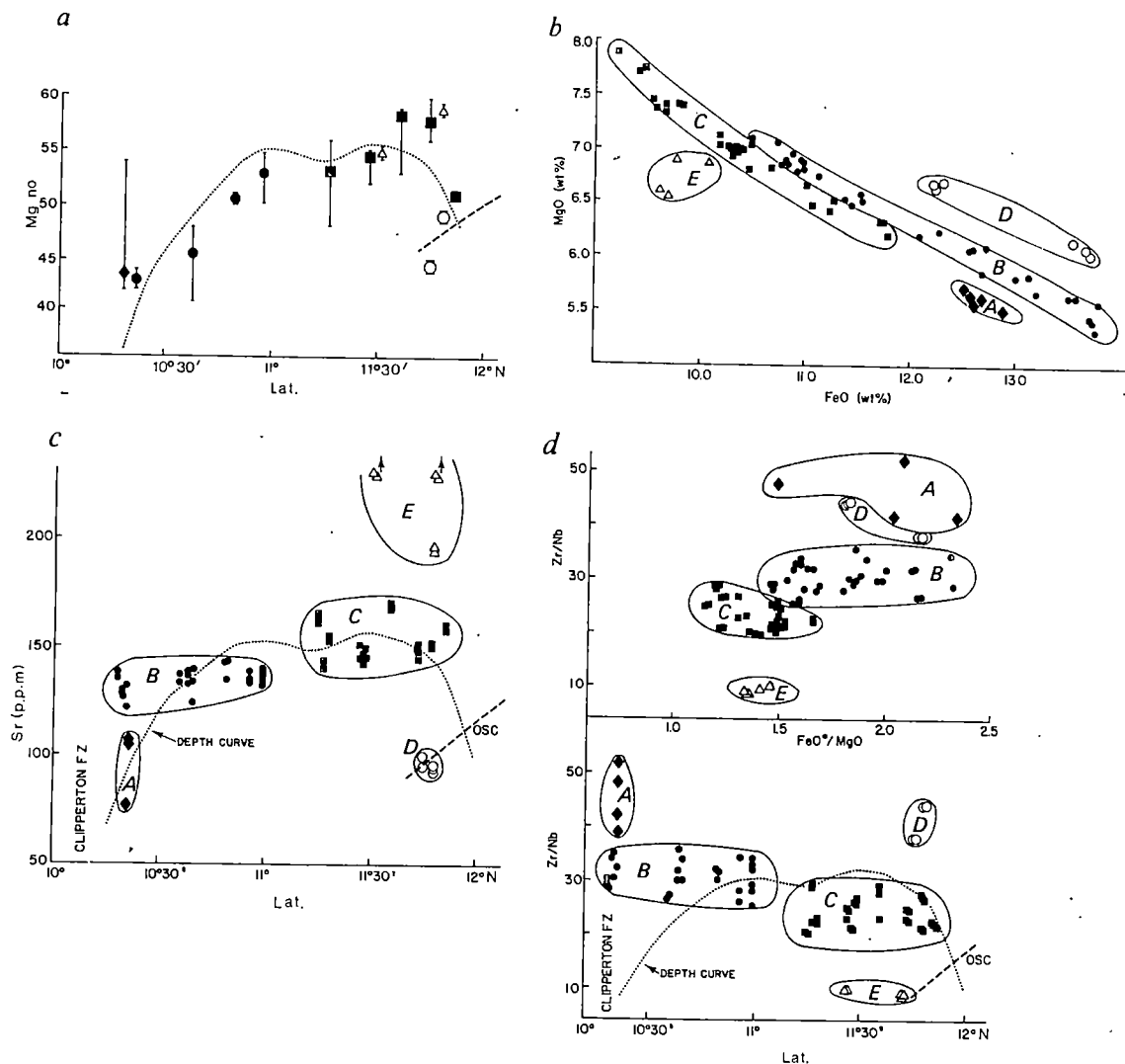


Fig. 3 *a*, Mg number ($\text{Mg}/(\text{Mg} + \text{Fe}) \times 100$) versus latitude along EPR axis 10–12° N. Symbols are mean Mg numbers, and the vertical line shows the range of Mg numbers at that location. *b*, MgO versus FeO for basalt glasses from the EPR axis. Five different groupings are noted as well as the range of FeO/MgO, indicating the degree of fractionation. *c*, Sr versus latitude for basalts from EPR axis. Five distinct magma types and their location are distinguished. *d*, Zr/Nb versus FeO*/MgO and latitude for basalts from EPR axis. ♦, A, near Clipperton Fracture Zone; ●, B, South axis and topographic high; ■, C, north axis, topographic high and west OSC; ○, D, eastern OSC; △, E, E-MORB. The dotted line in *a*, *b*, and *d* is the axial depth (see Fig. 1*b*).

and have referred to this as the “transform edge effect” (TFE). We are unsure without further analysis (such as isotopes, rare-earth elements, Ta, Th, and so on) whether magma type A is another example of a TFE or merely an older axial eruptive event unrelated to the present axial eruptives represented by magma type B.

Magma type B shows a wide range in degree of fractionation which decreases systematically towards the topographic high. This is in agreement with the model suggesting major input to a master magma chamber beneath the topographic high. Lateral injection along-ridge (probably sub-crustally) from the chamber with concomitant crystallization as the magma moves into cooler crust, is a reasonable explanation which is also consistent with recent observations in Iceland¹². All the basalts from magma type B are normal MORB, as are those from magma type C from the second topographic high. Single specimens from either magma probably could not be discriminated as different types, but the large number of samples analysed here with high precision show small but consistent differences in both chemistry and modal phase proportions between the two main magma types B and C which persist across substantial variations in fractionation. Mineralogically, magma type B has plagioclase and pyroxene with little olivine, whereas magma type C has both olivine and plagioclase as phenocrysts but little pyroxene. Magma type B generally has higher Fe and lower Ti content for a given MgO concentration, and also has lower Sr contents,

lower Zr/Y ratios and higher Zr/Nb ratios compared with magma C (Fig. 3*b–d*). The differences between magma B and C are small and compositional ranges overlap. However, comparisons of trace-element ratios versus Mg number or FeO/MgO ratio show that the magmas are distinctly different in composition.

The topographic low between the axial highs is apparently filled with type C lavas, suggesting that the southward propagating limb of the northern high has almost closed this small OSC. The northernmost limb of the rift containing magma type C also becomes increasingly fractionated but as the distances are not so great, and the tip of this limb is apparently not active, the degree of fractionation is not so pronounced as on the long southern limb of the first topographic high of magma type B.

The magmas from the active eastern limb of the OSC, magma type D, have quite unique compositions compared with the main lavas of types B and C; they resemble group A in some but not all features. They have higher Fe and Ti contents for a given MgO concentration, low K and Sr content, low Zr/Y and high Zr/Nb ratios. They are phryic basalts in which olivine is a prominent phenocryst and include some varieties which are quite fractionated, but we are unsure whether they are fractionated extremities of lavas from the main chamber near 12°30' N, or lavas showing a TFE effect since they are at the tip of a propagating rift breaking into old, colder crust¹³.

Magma type E lavas, the so called E-MORB, were recovered

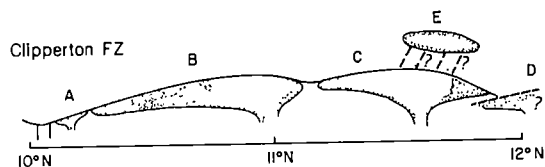


Fig. 4 Five magma types observed along EPR axis 10°–12° N. Dotted areas indicate >8% mineral phenocrysts. Magma type E is presumed to be an off-axis (4 km) seamount from which lavas have intruded into the axial region at two locations. It is uncertain whether magma type D is a unique magma batch like magma type A, near the propagating rift tip, or the proximal tip of a down-axis injection from a magma chamber near 12°30' at the next axial topographic high.

at two localities on the second topographic high. A previous cruise by the French *N.O.J. Charcot*⁷ in the same general area also recovered samples of E-MORB from the axial graben and from the eastern rim of the axis near 11°27' N. We recovered two samples about 20 km away in the northern part of the axis approaching the tip of the west limb of the OSC. R. Hekinian provided splits of the French samples which we analysed; these are E-MORBs similar to those we recovered. The type E lavas have higher Ti and lower Fe content for a given MgO concentration compared with the other axial lavas. They have very high concentrations of incompatible elements such as K, Sr, Zr and Nb, and higher Zr/Y and lower Zr/Nb ratios than the other magma types. The *Charcot* surveys have shown that at this latitude, about 11°45' N, there is a large seamount only about 4 km to the west of the ridge axis which is erupting various types of lavas (R. Hekinian, personal communication). Available data are not sufficient to determine whether lavas from this seamount may have been injected transversely, or whether they flowed on the sea floor from the seamount to the present EPR axis.

Conclusion

Figure 4 shows diagrammatically the distribution of the five magma types. Our observations support the Francheteau-Ballard model of axial processes. We suggest that the topographic highs are the locus of magma upwelling into magma

chambers, and that magmas are injected down the axes from these localities. These lavas show systematic changes in composition along the axis. In essence, the EPR is made up of a series of volcanoes separated by transform or non-transform (OSC) offsets. This is analogous to the observations of central volcanoes located along the rift zone in Iceland which have been shown to inject magma, subcrustally, up to 70 km along rifts from the central volcano; this injection often is highly asymmetric as in these EPR examples¹².

We have shown that the depression between the overlapping limbs of an OSC is old and inactive. We have demonstrated that the eastern limb of the OSC at 11°45' N is very active and propagating southward. Our data indicate that lavas at the propagating tip of an overlapping pair of rifts, or close to a large transform, may have distinct chemical compositions. Our observations also suggest local chemical heterogeneity in the mantle that must give rise to the pronounced differences between axial and nearby seamount lavas. We also observe small-scale mantle heterogeneity or differences in melt formation, local plumbing or magma mixing that give rise to the distinct compositions observed between individual magma centres or volcanoes along axis. These differences are superimposed on compositional variation which can be produced by the fairly extensive fractionation which has occurred within each magma batch. Further aspects of these differences and the nature of the source regions are currently being investigated by isotope and rare-earth element analysis and geochemical modelling. Although a variety of magma compositions have been noted in samples recovered previously from the EPR, this is the first documentation of systematic variation in lava composition and morphology that can be ascribed to specific axial processes and their relative position in time and space.

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Patterns of *engrailed* and *fushi tarazu* transcripts reveal novel intermediate stages in *Drosophila* segmentation

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Transcripts of the engrailed and fushi tarazu genes in young Drosophila embryos are initially patterned in intervals larger than a single segment. The segmental pattern evolves in a stepwise sequence through successively smaller spatial units.

THE *Drosophila* embryo is spatially organized into repeating units—the anterior and posterior developmental compartments¹ of each metameric segment. These compartment types alternate in a striking zebra-like pattern as transverse encircling bands of cells on the surface of the young embryo^{2,3}. Their presence is

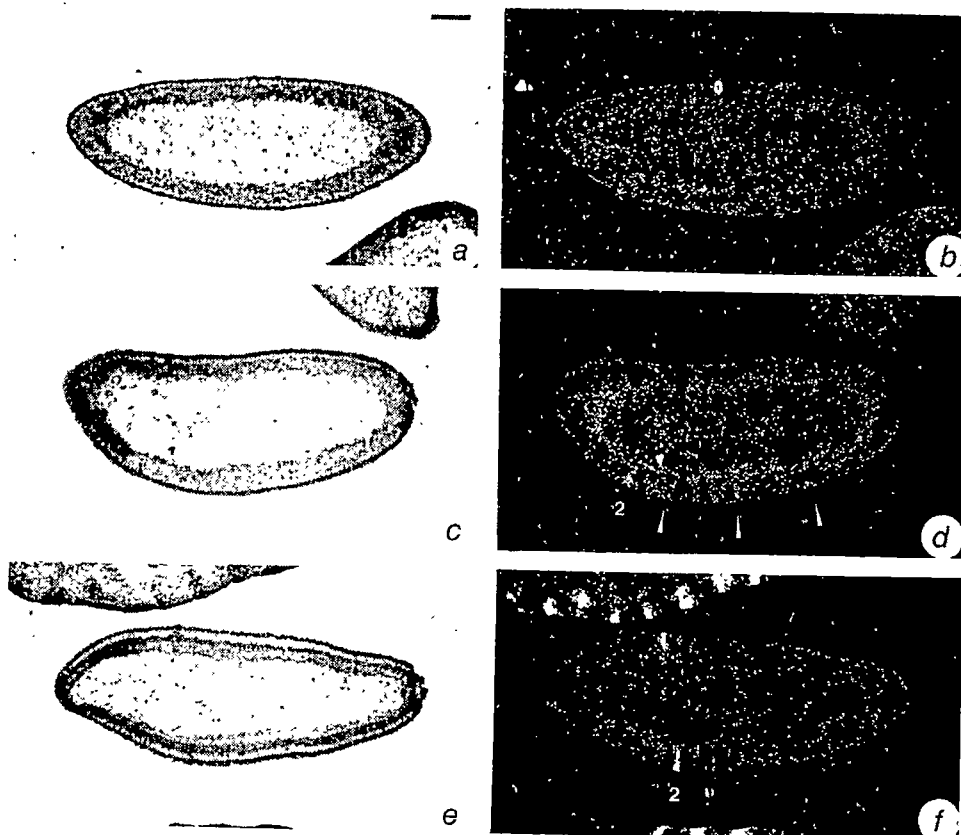
not apparent from the embryo morphology, but is revealed by cell lineage studies^{4–6}, and by *in situ* identification of cells that express the *engrailed* locus. The *engrailed* locus is expressed only in the posterior compartment cells².

The *engrailed* gene is one of a number of genes whose function

Fig. 1 Micrographs showing *engrailed* transcripts in blastoderm stage embryos. Bright-field (left) and corresponding dark-field (right) micrographs were recorded after hybridization with an *engrailed* cDNA probe and autoradiography. Sections in all figures are oriented anterior left. *a, b*, Section of embryo at nuclear cycle 13 with no detectable localization of autoradiographic grains. *c, d*, Early cycle 14 embryo, oriented ventral down, with nuclei beginning to elongate. Ten such sections were found with grains most prominently over the nuclei that later will correspond to the second of the 14 stripes (Fig. 2*f*). In this section, sparse concentrations of grains are also over the nuclei of what are probably stripes 4, 8 and 12 (arrowheads). *e, f*, Horizontal section. The nuclei have elongated and membrane furrows have extended into the cytoplasm just beyond the nuclei. A prominent autoradiographic signal is over the nuclei and cytoplasm of stripe 2. Scale bar, 50 μ m.

Methods. Embryos were dechorionated with 2.5% sodium hypochlorite for 3 min, fixed in 2% paraformaldehyde, 0.5 \times phosphate-buffered saline (PBS) and 50% *n*-heptane for 15 min using vigorous shaking, and transferred to 45% methanol, 25 mM

EGTA, 50% heptane and shaken on dry ice for 10 min. Vitelline membranes were then removed by rapid heating to 40 $^{\circ}$ C²⁷. Following three washes in 90% methanol, 50 mM EGTA, embryos were transferred in stages to PBS, before embedding in O.C.T. (Miles Scientific) using liquid nitrogen. *In situ* hybridizations onto 8 μ m frozen sections were performed as described previously using tritiated nick-translated DNA probes of the p9-1.4 cDNA². Embryonic stages: Observation during nuclear cycles 10-13, syncytial blastoderm stages, shows that at cycle 10, yolk nuclei stop dividing, pole cells bud from posterior end, and remaining nuclei appear on the surface and divide in near synchrony. At cycle 14, cellular blastoderm stage, there is a ~90 min period of mitotic quiescence during which membranes form between the peripheral nuclei and gastrulation begins. Embryos in the sections were staged by counting nuclei in 100- μ m intervals along the embryo periphery. 43 measurements of cycle 14 embryos yielded a value of 15.6 ± 1.4 nuclei per 100 μ m. The values used for other stages were: cycle 10, 4 nuclei; cycle 11, 6 nuclei; cycle 12, 8 nuclei; and cycle 13, 11 nuclei.



is essential for segmentation. The gene *fushi tarazu* (*ftz*) is another⁷, and it is expressed in the young embryo in seven evenly spaced bands of cells with a two-segment periodicity⁸. Although each band has the width of one segment⁹, the spatial relationship between the bands of *ftz*-expressing cells and the metameric segments is not known. Nevertheless, the expression of *engrailed* and *ftz* both provide indications of the timing and nature of the processes that subdivide the embryo into equal-sized metameres, and they provide molecular markers that can be used to follow the process of segmentation.

In this study, the generation of the *engrailed* and *ftz* patterns of expression have been determined by *in situ* hybridization. The use of fixation methods that better preserve the morphology of the early *Drosophila* embryo and longer autoradiographic exposures have yielded a more precise portrayal of the generation of the segmental patterns and have revealed several new aspects of segmentation. Transcription patterns for both *engrailed* and *ftz* suggest that subdivision of the embryo proceeds through a sequence of intermediate stages, each stage part of a process that partitions the embryo into progressively smaller units.

Expression of *engrailed* in early embryos

After fertilization and nine synchronous mitotic divisions, three types of nuclei can be distinguished in the *Drosophila* (cycle 10) embryo: approximately 100 centrally located yolk nuclei, about 450 peripheral nuclei at the embryo surface that will generate the somatic tissues, and about 12 future germ cells at the posterior pole¹⁰⁻¹⁴. Four divisions of the peripheral nuclei follow. During the 14th cell cycle, the nuclei, initially round,

elongate before membrane furrows invaginate from the surface of the embryo to separate the nuclei into individual cells¹⁵. During this period the nuclei become highly active in transcription^{16,17} (A description of relevant embryonic stages is given in Fig. 1 legend.)

To identify nuclei that express the *engrailed* locus, wild-type embryos were fixed and quick-frozen for preparation of cryostat sections. After hybridization with radiolabelled probes generated from an *engrailed* cDNA clone², autoradiographs were exposed for 3-4 months to detect *engrailed* transcripts. Sections from embryos younger than stage 14 did not have grains localized either over nuclei or in any other distinctive pattern (Fig. 1*a, b*), yet the grain density over such embryos was consistently higher than background. Since Northern blot analysis of RNA isolated from cleavage stage and syncytial blastoderm stage embryos has revealed the presence of *engrailed* transcripts¹⁸, these early transcripts are either not regionally localized or our *in situ* hybridization methods lacked sufficient sensitivity for definitive detection.

As the embryo matures through the 14th division cycle, striking changes in *engrailed* expression produce a pattern of 14 equally spaced and equally dense stripes (Fig. 2*f*). The pattern is generated in a reproducible and recognizable sequence. Anterior bands appear before posterior ones, ventral bands before dorsal ones (Figs 1, 2). However, superimposed on this anterior to posterior sequence of appearance are strong but transient periodicities in the density of autoradiographic grains over the different bands: (1) the first two prominent bands in the ventral region are bands 2 and 8; (2) bands 4 and 8 become prominent before band 6, and band 12 before 10, suggesting a

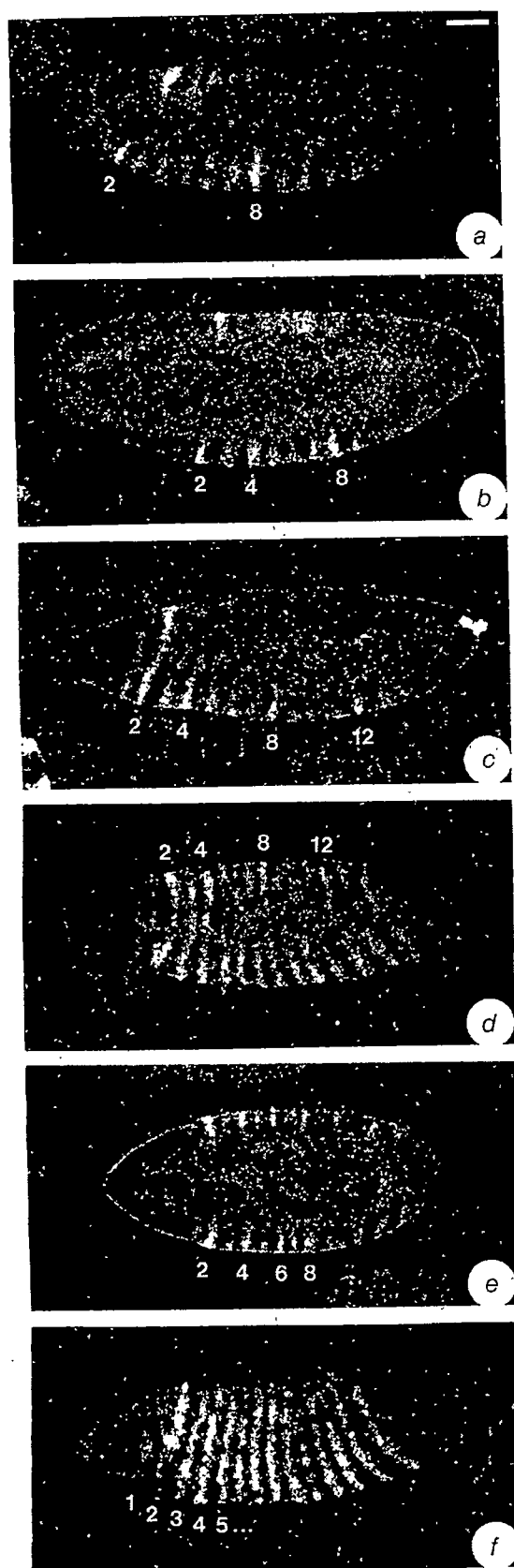


Fig. 2 Micrographs of *engrailed* transcripts in early gastrulas. Dark-field micrographs of embryos oriented ventral down except for *e*, a horizontal section. Stripes are more prominent in the anterior and ventral portions. Most prominent stripes are: *a*, ventral 2 and 8, dorsal 2; *b*, ventral 2, 4 and 8, dorsal 2; *c*, ventral 2, 4, 8 and 12, dorsal 2; *d*, ventral 2-14 and dorsal 2, 4, 8 and 12; *e*, lateral 2, 4, 6, 8, 10, 12; *f*, all stripes approximately equal. Note that among the weak stripes between 4 and 8 in *b*, stripe 6 is the weakest. *a* Has a two-band pattern, *b*-*d*, four-segment periodicity, and *e*, a two-segment periodicity. Scale bar 50 μ m.

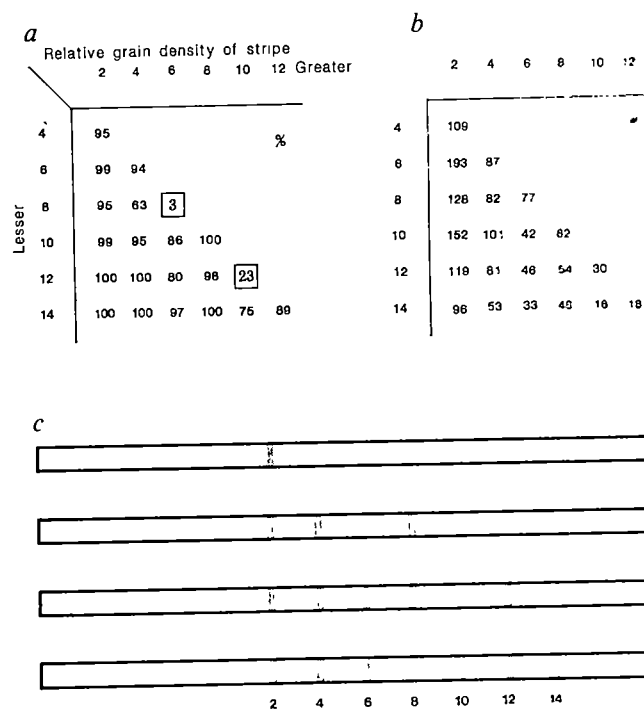


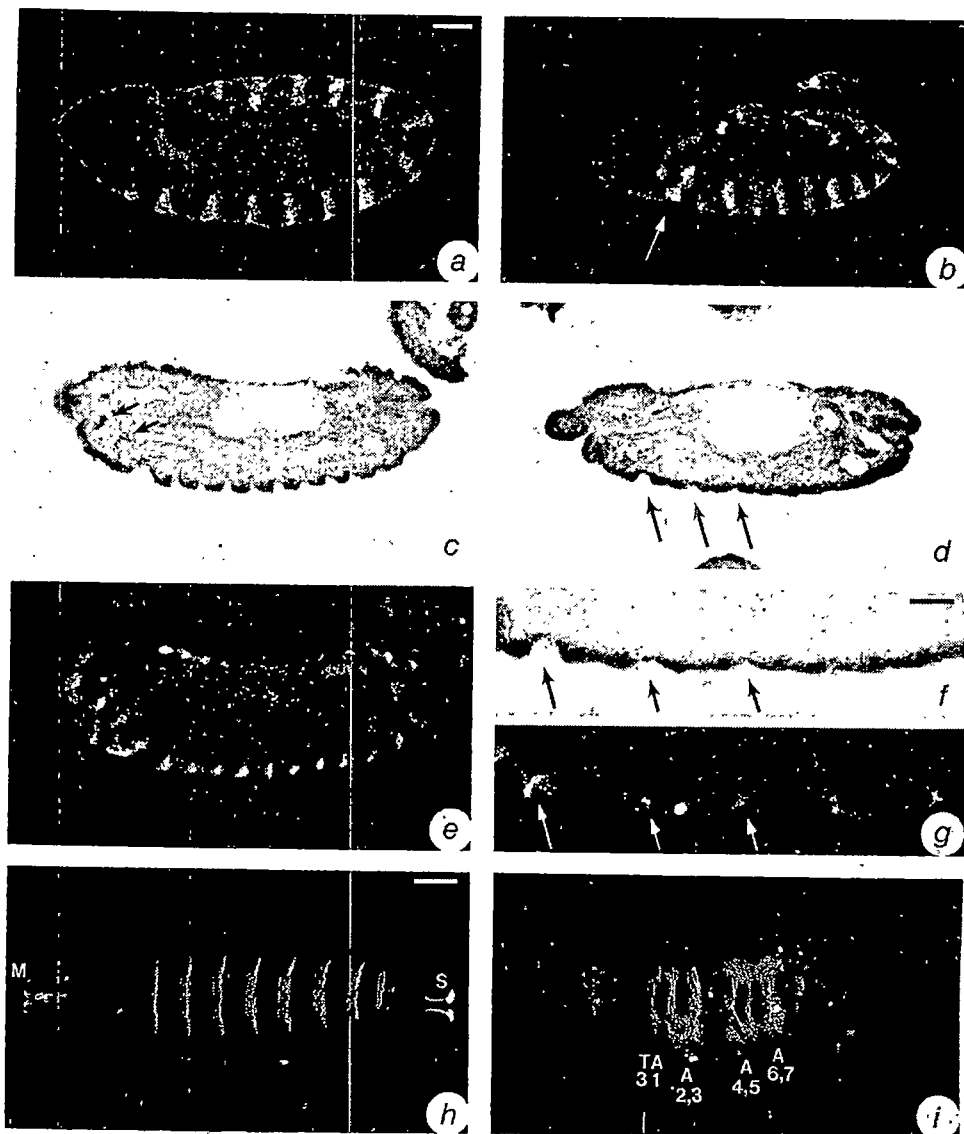
Fig. 3 The order of appearance of the even-numbered bands of *engrailed* hybridization was determined in 247 section edges from 132 sections of 105 different embryos. Section edges chosen for examination were those with clear differences in band intensities. After a pairwise comparison of each of the even-numbered bands, only cases of clear intensity differences were tabulated. *b*, Total number of instances in which two bands with different intensities were compared; *a*, the percentage of such comparisons in which the bands designated along the horizontal axis were more intense than those indicated on the vertical axis. The boxed numbers indicate cases of posterior stripes appearing before anterior ones: 8 before 6 and 12 before 10. *c*, A summary of the types of patterns that were observed. Embryos of increasing age are portrayed as rectangles with their posterior pole to the right. The dotted areas indicate bands of hybridization.

four-segment reiterated pattern; and (3) when all of the even-numbered bands become prominent, they have autoradiographic signals that are briefly stronger than those of the intervening odd-numbered bands, suggesting a two-segment reiterated pattern. A detailed description of this progression follows.

The first of the prominent bands of hybridization was observed in early cycle 14 embryos whose nuclei had just initiated the elongation process (Fig. 1*c, d*). A stripe of grains with a width of one to two nuclei was oriented perpendicular to the long axis of the embryo, about one-third from the anterior end. Grain density was initially greater over the nuclei than over the adjacent cytoplasm (Fig. 1*c, d*), but during the 14th cycle, the relative proportion of cytoplasmic grains increased (Fig. 1*e, f*). With the commencement of gastrulation near the end of cycle 14, the stripe of hybridization was at the posterior edge of the cephalic furrow, and corresponded to the second of the 14 stripes from the anterior end (Fig. 2*f*). The posterior edge of the cephalic furrow is the position of the mandibular or maxillary head segment primordium^{9,19}.

The fourth and eighth bands, weak concentrations of grains at the beginning of cycle 14 (Fig. 1*c, d*), were (along with band 2) consistently the most intense as gastrulation began (Fig. 2*a-d*). The relative strengths of the autoradiographic signals over the fourth and eighth stripes were often unequal (82 cases). In such sections in which the ventral and dorsal surfaces could be unambiguously identified, band 4 was more intense than band 8 dorsally (19/19 cases), and band 8 was more intense ventrally (13/16 cases) (Fig. 2*a*). Thus, ventrally, band 8 appeared before 4, and dorsally, band 4 appeared before 8. Band 12 was also observed as a prominent early autoradiographic signal (Fig. 2*c, d*), although with less regularity.

Fig. 4 Micrographs of *engrailed* transcripts in *engrailed* mutants. *a*, Dark-field micrograph of a section of a gastrulating wild-type embryo. The grains of adjacent stripes are approximately equal in density (also see Fig. 2*f*). *b*, Dark-field micrograph of a section of an *en^{C2}/en^{SF31}* mutant embryo during germ-band elongation (*en^{SF31}* deletes the entire *engrailed* locus). Signal alternates in strength in adjacent segments and the phasing is the same as observed in younger wild-type embryos: prominent stripe 2 is located in the posterior part of the cephalic furrow (arrow). *c*, *e*, Bright-field and dark-field images of a wild type embryo following germ-band shortening. The signal strengths are approximately equal over adjacent segments. Note the grains over the anterior portion of the ventral nerve chord (arrows in *c*). Serial sections of the embryo revealed labelling over the other ventral nerve chord ganglia. Such expression is consistent with earlier reports that *engrailed* function is essential in the adult nervous system²⁸. *d*, Bright-field micrographs of a section of an *en^{LA4}/en^{LA7}* mutant embryo. Pairs of segments are fused such that deep grooves are only observed every second segment (arrows). *f*, *g*, Higher magnification views of the periphery of the mutant in *d*. Grains are over every second segment over the deep grooves (arrows). Few or no grains are over the sites of the segment fusions. *h*, Dark-field micrograph of the ventral cuticle of an 18-h wild-type embryo. Note the mouthparts (M), eight abdominal denticle bands, and posterior spiracles (S). *i*, Dark-field micrograph of the ventral cuticle of an *en^{LA4}/en^{SF31}* embryo. Fused abdominal denticle belts are indicated. Scale bars, 50 μ m in *a-e*; 25 μ m in *f*, *g*; and 100 μ m in *h*, *i*.



In 18 sections with intense labelling in bands 4 and 8 or 8 and 12, the strengths of the bands in the three-band interval between them varied such that the intervening odd-numbered bands were more intense than the even-numbered ones (Fig. 2*b*).

To document the order of appearance of these even-numbered bands, sections whose bands varied in intensity were examined. In 132 such embryo sections, pairs of even-numbered bands were compared and instances of clear differences in intensity were scored (Fig. 3). Band 2 was almost always more intense, and with only two exceptions, the more anterior bands were more intense than each of the posterior bands. As exceptions, the 8th was usually more intense than the 6th and the 12th more than the 10th (Fig. 3*a*, *b*). A pictorial representation of embryos with the implied four-segment periodic patterns is presented in order of increasing complexity and maturity (Fig. 3*c*, see also Figs 1*d*, 2*b-d*).

Even-numbered autoradiographic stripes increased in intensity throughout the 14th cycle. Between these were stripes with less intense signals. The resulting pattern, most prominent at the beginning of gastrulation, had a two-segment periodicity with alternating strong and weak autoradiographic signals over adjacent segments (Fig. 2*e*). The autoradiographic grains of the more intense even-numbered bands were dense over both the nuclear and cytoplasmic regions; in contrast the less intense odd-numbered bands were predominantly labelled over the nuclei.

The alternating pattern leads within minutes to an arrangement of stripes with equal intensity. The equalization process

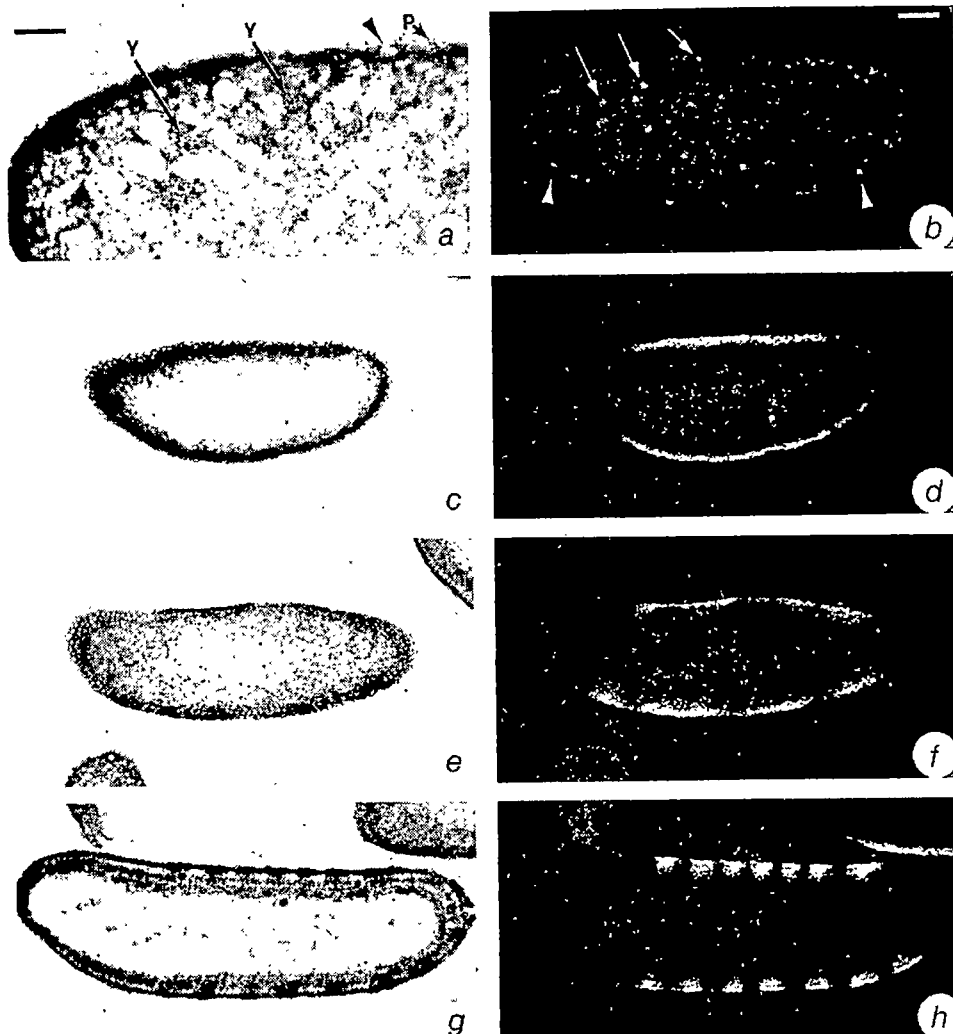
occurred with an anterior to posterior and a ventral to dorsal progression. As gastrulation proceeds, the ventral germ band extends around the posterior end onto the dorsal surface. In sections of germ band elongation embryos, additional bands of hybridization in the head and posterior regions were observed as reported previously². By this stage, all of the stripes were two to three cells wide and were equally intense. Consistent with previous descriptions, cells expressing the *engrailed* locus were observed in the progenitors of the mesoderm and nervous system throughout the germ-band elongation stages. However, the improved preservation of sections and longer exposures revealed, in addition, expression in each segmental ganglion of the embryonic nervous system during later stages (Fig. 4*c*).

Expression of *fushi tarazu* in early embryos

Gene expression in a pattern with reiterated periodic bands was first described for the *fushi tarazu* locus⁸. To compare the processes that generate the patterns of *engrailed* and *ftz* expression, embryo sections were hybridized with a *ftz* probe in parallel to the experiments described above for the *engrailed* gene. Our results largely confirm the work of Hafen *et al.*⁸, but reveal two additional intermediate stages in the temporal and spatial pattern of *ftz* expression—generally dispersed transcription at nuclear cycle 10 and a four-band stage early in cycle 14.

Localization of *ftz* RNA was first detected in syncytial blastoderm embryos during their 10th nuclear division cycle. Autoradiographic grains were localized over both yolk and

Fig. 5 Patterns of *fushi tarazu* transcripts in syncytial and cellular blastoderm stage embryos. *a, b*, *ftz* transcripts were detected over peripheral (P) and yolk nuclei (Y) at cycle 10 (arrows in *a* and *b*). The bright-field micrograph is a high-magnification image of the section shown in dark-field in *b*. Arrows point to corresponding nuclei. Arrowhead in *a* points to an unlabelled nucleus. Arrowheads in *b* point to examples of labelled peripheral nuclei. *c, d*, Embryo at cycle 12. During cycles 11–13, *ftz* transcript is evenly distributed over the middle portion at the edge of the embryo as previously reported⁸. *e, f*, Cycle-14 embryo; *ftz* transcripts are in four broad bands. *g, h*, Cycle-14 embryo with membrane furrows extended just beyond the nuclei; *ftz* transcripts are in seven bands. The probe was pDmV61H3.5 (a genomic 3.5 kilobase pair *Hind*III subclone in pUC8²⁹), nick-translated with tritiated nucleotides. Scale bar, 25 μ m in *a*, and 50 μ m in *b–h*.



peripheral nuclei (Fig. 5*a, b*). Labelled nuclei were along the entire egg length, although not all peripheral nuclei were labelled. By the 12th cycle, *ftz* RNA was uniformly distributed in the cortical region of the embryo, from approximately 15% to 65% egg length (Fig. 5*c, d*). No further regional localization of the hybridizing RNA was observed until the 14th cycle, when autoradiographic grains were in discrete regions that approximate four broad bands (Fig. 5*e, f*). The most anterior band was not as wide as the others, but as the nuclei elongate and before the membrane furrows penetrate into the embryo, the three posterior broad stripes subdivided and yielded a pattern of seven discrete regions of hybridization (Fig. 5*g, h*). At both the four- and seven-band stages, the discrete gaps that define the bands appeared in an anterior-to-posterior and ventral-to-dorsal progression. There was no apparent difference in density of autoradiographic grains over the different stripes. In stage 14 embryos *ftz* transcripts were more numerous than *engrailed* as estimated from grain densities after *in situ* hybridization.

The existence of the transient four-band stage was documented by tabulating the order of appearance of gaps between hybridization stripes. With only three exceptions, the gaps defined the more anterior stripes first. These exceptions were: the gap between stripes 3 and 4 formed before the stripe 2–3 separation, and the gap between stripes 5 and 6 formed before the stripe 2–3 and stripe 4–5 separations (Fig. 6). Figure 6*c* summarizes the implied progression of *ftz* expression from uniform to banded distribution.

To establish the relationship between the stripes of *ftz* and *engrailed* hybridization and the segment primordia in the blastoderm, the repeat lengths of the various periodic patterns were

compared. In sections of early stage 14 embryos in which the gaps between bands of *ftz* transcript were developing, the distance between gaps 1–2 and 3–4, and between gaps 3–4 and 5–6 was $88 \pm 11 \mu\text{m}$, corresponding to 13.8 ± 1.8 adjacent nuclei (56 measurements were taken). In sections of early stage 14 embryos hybridized with the *engrailed* probe, the distance between prominent bands 4 and 8, and between 8 and 12 was $92 \pm 11 \mu\text{m}$, corresponding to 14.4 ± 1.7 adjacent nuclei (46 measurements). Thus, the intervals of *engrailed* expression defined by bands 4, 8 and 12 have the same periodicity as the initial gaps of *ftz* expression. Given the estimated segment width of 3–4 cells⁹, this periodicity corresponds to four segments. As described previously^{2,3,8}, later cycle 14 embryos with 14 stripes of *engrailed* and 7 stripes of *ftz* expression have periodicities that correspond to one and two segments, respectively.

Throughout the syncytial blastoderm and cellular blastoderm stages, the autoradiographic grains of *ftz* hybridization were most concentrated over the periplasmic region between the nuclei and the embryo cortex. This sequestration of *ftz* RNA contrasts with the more generally dispersed *engrailed* RNA and may suggest novel mechanisms for the regulation of RNA storage and utilization.

Transcript localization in *engrailed* embryos

All developmental stages^{18,20–22} are affected by *engrailed* mutations. The *engrailed* cells in mosaic adults develop abnormally in all posterior compartments and generate similar types of defects in each segment^{20–24}. Yet mutant embryos have fused pairs of segments, and very occasionally, fusions of groups of four segments (Fig. 4*h, i*)²². A gene such as *engrailed* that is

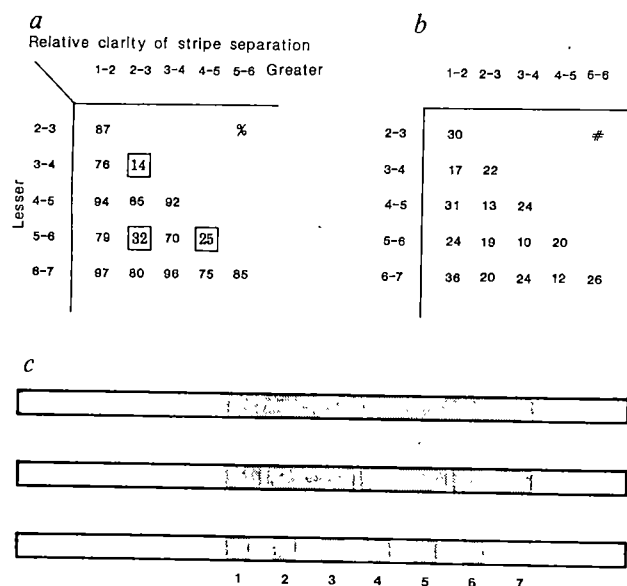


Fig. 6 The order of appearance of gaps in *ftz* expression along the embryo periphery was determined in 68 section edges from 34 sections. Sections chosen for analysis had gaps of varying clarity. For each comparison of two gaps, part *b* indicates the total number of cases with differences in the clarity of the gaps and part *a* indicates the percentages of such comparisons in which the gaps designated along the horizontal axis were more pronounced than the gaps on the vertical axis. The boxed figures indicate cases of posterior gaps becoming pronounced before anterior ones. *c*, A summary of the types of patterns observed. Embryos of increasing age are portrayed as rectangles with their posterior pole to the right. The dotted areas indicate regions of hybridization.

expressed in every segment might be expected to affect all segments similarly, whereas pairwise segment fusions might be expected in mutants of genes required in every second segment²⁵. Thus the *engrailed* embryonic phenotype was surprising. We now demonstrate a direct correlation between the fusion phenotype of *engrailed* mutant embryos and the expression of the *engrailed* gene in periodic, two-segment-wide intervals.

Sections of *engrailed* mutant embryos were hybridized with the *engrailed* complementary DNA probe. Mutants examined were *en*^{LA4}/*en*^{LA7} heterozygotes (both apparent point mutations), and *en*^{C2} (an inversion breakpoint within the *engrailed* locus approximately 15 kilobase pairs upstream of the transcription unit (B. Drees and T.K., unpublished)) hemizygotes²². Mutants were identified in sections by their abnormal morphology and abnormal distribution of *engrailed* transcripts. Whereas in wild-type embryos the intensity of hybridization to the posterior compartment stripes alternated for a brief period in the cellular blastoderm and early gastrula and was uniform thereafter (Figs 2*f*, 4*a*, *c*, *e*), in most mutant embryos the alternating intensity persisted through germ-band elongation and germ-band shortening (Fig. 4*b*, *d*, *f*, *g*). The identities of the more prominent stripes in the mutant embryos corresponded with the location of the more intense stripes of hybridization of younger wild-type embryos; for example, the stripe at the posterior edge of the cephalic furrow was prominent in both (arrow in Fig. 4*b*). Comparison of the phasing of the alternating transcriptional pattern with the fused segment morphology of the mutants indicated that the weaker stripes of the alternating pattern coincide with the positions of the segment fusions (Fig. 4*d*, *f*, *g*).

Thus, apparent point mutations or breakpoint mutations upstream of the transcription unit both affect the spatial and temporal pattern of *engrailed* expression. This demonstrates that DNA 5' to the transcription unit is involved in transcriptional regulation and that the *engrailed* function may modulate its own expression, either by directly affecting transcription or through interactions with other regulatory factors.

Discussion

The surface of the *Drosophila* cellular blastoderm is uniform in appearance, but its constituent cells are not equivalent. These studies and previous work⁸ have shown that during the 14th nuclear division cycle, the blastoderm cells differentiate to express selected genes in remarkably precise patterns. Maturation of the expression patterns of the *engrailed* and *ftz* genes have provided two views of this differentiation process, a process that progressively subdivides the embryo into biochemically and spatially distinctive portions.

Apparent differences between the development of the *engrailed* and *ftz* patterns of expression include earlier maturation of the *ftz* pattern and contrasting modes for generating bands of expression: new bands of *engrailed* expressing nuclei seem to be added while *ftz*-expressing nuclei are suppressed in a patterned arrangement. However, direct comparisons between the patterns of *engrailed* and *ftz* RNA are made difficult by the lower abundance of the *engrailed* transcripts. The presence of *engrailed* transcripts in cleavage and syncytial blastoderm stages has been demonstrated by Northern analysis¹⁸, but the low-level expression, which may be generally dispersed or distributed in a portion of the embryo, was not detectable with our *in situ* hybridization methods. The appearance of the *engrailed* patterns in *in situ* autoradiograms may be delayed until transcript levels are sufficient to be detected by our assay. Therefore, the great quantitative difference between levels of *ftz* and *engrailed* transcripts may mask fundamental similarities.

For both *engrailed* and *ftz*, the mature pattern of equally sized and equally spaced stripes of expressing cells emerges from patterns with longer repeat lengths that define fewer units. Maturation proceeds with anterior to posterior and ventral to dorsal polarities. For *ftz*, expression progressively narrows to four broad bands with a four-segment repeat length before dividing into seven narrower ones with a two-segment-repeat length. For *engrailed*, bands one to two cells wide define long intervals that shorten in a stepwise manner—first two bands (2 and 8 ventrally), then a four-segment periodicity, then two, and then in one-segment intervals.

That reiterated patterns of expression progress from longer to shorter repeat lengths suggests that the process of segmentation involves progressive partitioning of large units into smaller ones. The existence of discrete intermediate stages of segmentation has been proposed previously on theoretical grounds²⁶, and is strongly supported by the pattern of *engrailed* expression in *engrailed* mutants. Mutant late gastrulas had *engrailed* transcripts in patterns characteristic of younger embryos—in intervals greater than a single segment—indicating that the segmental fusions of *engrailed* mutants may be relics of earlier developmental units. A similar conclusion was drawn earlier from the phenotypes of the 'pair-rule' segmentation mutants that affect homologous portions of every second embryonic segment. It was suggested that such phenotypes are indicative of the transient existence of double-segment-sized units or fields²⁵.

The sequence in which the spacing of segments in the embryo is defined eliminates several possible mechanisms of segmentation. It indicates that the precisely spaced rows of cells in each segment are not designated in a simple sequential fashion at some predefined interval along the embryo's longitudinal axis. Nor do the many elements of the pattern appear simultaneously in their final form. Rather the mature pattern seems to involve proportioning of the embryo into successively smaller units. Such proportioning may provide the basis for coding position along the embryo's longitudinal axis. The overlapping activities of genes expressed with periodicities of one segment, two segments and larger intervals, together could direct in a combinatorial way the development of the blastoderm cells.

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Transcription pattern of the *Drosophila* segmentation gene *hairy*

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Segmentation of the Drosophila embryo requires expression of the pair-rule genes, mutations of which cause reiterated deletions in alternate segments along the antero-posterior body axis. We find that transcripts of one such gene, hairy, accumulate in eight distinct regions of the early embryo. This pattern of expression is compared with that of another pair-rule gene, fushi tarazu, and its dependence on maternally expressed genes is described.

A COMMON theme in the organization of many metazoa is the subdivision of the body into a series of homologous segments¹. Different structures are then elaborated in the various segments to generate complex forms. A vivid example of such segmental or metamer organization is provided by the insects. Although segmentation of the insect body has been studied for many years²⁻⁴, the prospect of an understanding at the molecular level has only recently come into view with the identification, in *Drosophila melanogaster*, of most of the genes required for the process⁵⁻⁹.

Metameric organization is established during early *Drosophila* embryogenesis¹⁰⁻¹² as the incipient cells of the blastoderm embryo respond to information generated epigenetically in the maternally derived environment of the egg^{13,14}. This response depends on the expression of the zygotic genome and, in particular of genes defined by a class of zygotically acting mutations⁵⁻⁸ which disrupt the generation of the normal segmental pattern. These fall into three distinct phenotypic classes: (1) the gap mutations, which cause the deletion of contiguous groups of segments, (2) the pair-rule mutations, which result in deletions of homologous pattern elements in alternate segments, and (3) the segment polarity mutations, which affect the patterning of every segment.

The discovery of the pair-rule class provided the first evidence of a double-segment order during the formation of the metameric pattern⁵. This order is reflected at the molecular level by the pattern of expression¹⁵ of the pair-rule gene *fushi tarazu* (*ftz*)^{16,17}. At the blastoderm stage, *ftz* transcripts accumulate in seven discrete regions which repeat along the antero-posterior axis of the embryo with double-segment periodicity¹⁵. Such a precise pattern of transcript distribution implies that the *ftz*⁺ function is specifically required for the establishment of particular metameric units—those which are deleted in *ftz*⁻ mutants. Mutants of a second pair-rule gene, *hairy*(*h*)^{5,18}, cause a pattern of deletions which is approximately complementary to that typical of *ftz*, suggesting a corresponding complementarity of spatial expression and function.

We have analysed the spatial distribution of *h* RNA in early embryos, and compare it with the previously described *ftz* transcription pattern. We also show that establishment of the *h* transcription pattern depends on maternal determinants of both antero-posterior and dorso-ventral polarity.

Effect of *hairy* on embryonic segmentation

The metameric organization of the *Drosophila* body is directly reflected in the cuticle of the first-instar larva. This is especially clearly illustrated by the pattern on its ventral surface (Fig. 1 and ref. 19). Each thoracic and abdominal segment secretes a characteristic belt of cuticular processes or denticles. The metameric origin of the mouthparts is obscured by head involution, but cuticular structures characteristic of each segment can be easily identified.

Larvae homozygous for *h* null mutations exhibit a range of segmentation defects¹⁸ representing variations on a common theme, the pair-rule phenotype. The most regular *h* phenotype (Fig. 1c) consists of the deletion of the anterior region of each even-numbered abdominal segment and the posterior region of each odd-numbered abdominal segment from the larval cuticular pattern. This is almost the precise complement of the phenotype characteristic of amorphic alleles of the pair-rule gene *ftz* (Fig. 1d). In both cases, the same register of deletions extends into the thoracic and head segments (Fig. 1a). In *h* mutants, structures which derive from the mandibular (G1) and labial (G3) segments (the ventral arms of the cephalopharyngeal skeleton and H-piece respectively)²⁰ are deleted. In addition the medial tooth, a derivative of the labrum, is disrupted. This contrasts with the phenotype of strong *ftz* alleles in which only derivatives of the post-oral maxillary and labial segments are affected.

This regular pair-rule phenotype is typical only of a proportion of animals homozygous for any given amorphic *h* allele¹⁸. Commonly, more extreme defects are seen. The entire third thoracic segment is often deleted such that the second thoracic and first abdominal denticle bands fuse; similarly the remaining three posterior abdominal bands frequently fuse to form a continuous 'lawn' of denticles exhibiting polarity perturbation^{18,21}.

These lesions in the larval cuticle are manifestations of defects occurring much earlier during embryogenesis. Absence of *h*

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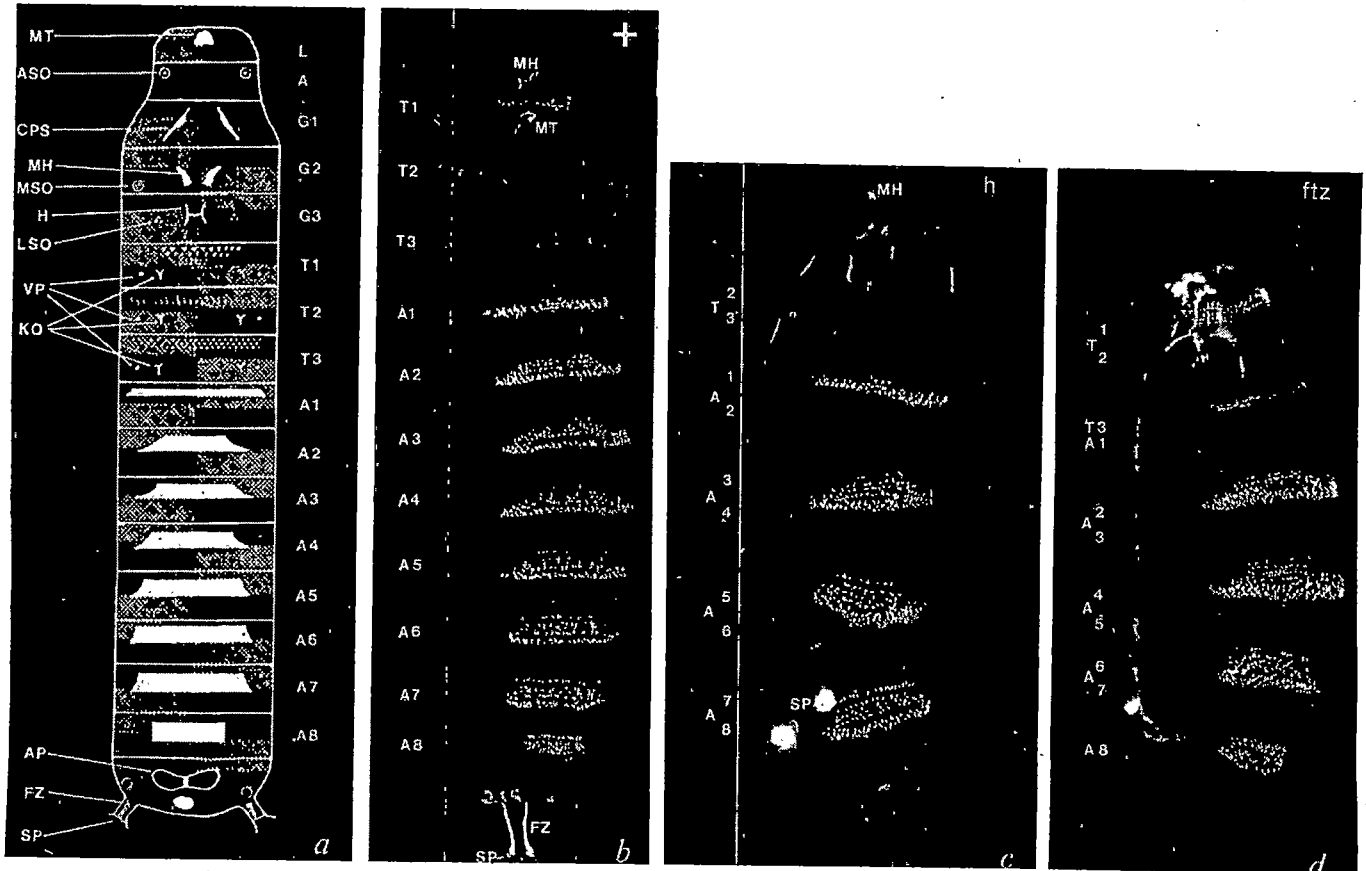


Fig. 1 *a*, Schematic representation of a first-instar *Drosophila* larva. The cephalon and mouthpart segments are drawn as they would appear in the absence of head involution. Each thoracic and abdominal segment is characterized by a ventral denticle band, represented by white bands. The thoracic segments each bear a pair of ventral pits (VP) and Keilin's organs (KO) which probably mark the position⁴⁰ of the antero-posterior compartment⁴¹ boundary. The thoracic segments each differentiate specific structures: LSO, labial sense organs; H, H-piece; MSO, maxillary sense organs; MH, mouth hooks; CPS, cephalopharyngeal skeleton. Anterior to these are the antennal sense organs (ASO) and the medial tooth (MT) of the labrum (see ref. 20). The hatched regions represent the consensus deletions typical of either *h* (left) or *ftz* (right) mutations (see *c* and *d*). *b*, Ventral cuticle of a first-instar larva viewed under dark-field illumination, showing the thoracic (T1-T3) and abdominal (A1-A8) denticle bands. *c*, Larvae homozygous for a deficiency or null mutations of *h* exhibit a reduction in the number of ventral denticle bands. Specifically, most of the denticle bands of each even-numbered abdominal segment (A2, A4, A6, A8) are deleted. In addition the naked posterior parts of each odd-numbered segment (A1, A3, A5, A7) are deleted. This frame of deletion extends into the thoracic and gnathal segments; in addition the medial tooth, a derivative of the labrum, is disrupted. The precise limits of the deleted regions are indicated in the left-hand half of the schematic larva shown in *a*. These have been deduced from the study of partial deletions in weaker genotypes (P.W.I. *et al.*, in preparation) and, in the case of the A1/A2 deletion, by using the *Ultrathorax* (*Ubx*) mutation to mark anterior A1 derivatives²³. The deleted region in larvae exhibiting this regular pair-rule phenotype varies in size but is approximately the same width as a segment. It includes the segment boundary, its limits being anterior to the presumed position⁴⁰ of the anterior/posterior compartment boundary in each segment. *d*, Larvae carrying null mutations of *ftz* (*ftz*^{W20}/*ftz*^{9H34})^{7,16} exhibit a phenotype which is the reciprocal of that typical of *h* mutants. Thus the anterior of each odd-numbered and the posterior of each even-numbered abdominal segment is deleted. This deletion frame similarly extends into the thoracic and gnathal segments and is shown schematically in the right-hand half of *a*. Our assessment of the *ftz* phenotype differs in several respects from previously published descriptions^{16,17}. For instance we find the H piece, a derivative of the labial segment, to be present, in contrast to the assertion of Wakimoto *et al.*¹⁶ (but see their Fig. 6d which clearly shows an H piece). We also consider the boundaries of the deletion frame to lie, like those of *h*, anterior to the anterior/posterior compartment boundary. This is supported specifically by three observations: (1) The secondary patch of denticles in T1 which lies posterior to the major denticle band but anterior to the Keilins organs (which mark the anterior/posterior boundary)⁴⁰ is partially deleted in *ftz* larvae. (2) The Keilin's organs posterior to the T3 denticle band are usually deleted. (3) Part of the salivary gland which derives from the anterior compartment of G3 is retained in *ftz* mutants (P.W.I., K.R.H., M. E. Akam and A. Martinez-Arias, in preparation).

has a clearly visible effect within 1 h of the cellular blastoderm stage when the germ band fails to extend fully (data not shown). By the time the parasegmental grooves²² are visible (Fig. 2*a*) the metameric organization is obviously disturbed, the germ band being divided into, at most, only half the normal number of parasegmental units (Fig. 2*b*). At the same time, discrete regions of cell necrosis become evident along the length of the germ band (Fig. 2*c*).

hairy RNA during early embryogenesis

Several different transcripts derive from the *h* gene (ref. 23 and K.R.H., in preparation). During embryogenesis two species can be detected by Northern blot hybridization: an RNA of about 2.1 kilobases (kb), denoted α_L , and, at lower levels, a slightly smaller (~2.0 kb) transcript, α_S . The expression of the *h* α transcripts during early embryogenesis has been analysed by *in situ* hybridization of a 1.7-kb genomic probe to wax-embedded sections. Hybridization to adjacent sections with *ftz* probe allows a direct comparison of the expression of both genes in the same embryos (see Fig. 3).

Both *h* and *ftz* transcripts are first detectable during the beginning of the interphase two cycles before cellularization of the blastoderm (stage 12)²⁴. Whilst *ftz* expression is restricted to a specific region, *h* transcripts are more or less uniformly distributed throughout the egg (Fig. 3*d-f*). Subsequently, two distinct regions of *h* transcript accumulation can be detected. Anteriorly, labelling is restricted to the dorsal half of the embryo in a region between 95% and 85% egg length, whilst a second domain of continuous labelling around the entire circumference of the embryo extends from approximately 75% to 20% egg length. Labelling in this latter domain rapidly becomes discontinuous (Fig. 3*g-i*); by mid-stage 14, prior to the completion of cellularization, *h* transcripts are localized in eight distinct regions along the antero-posterior axis of the embryo (Fig. 3*j-l*). The most anterior of these (designated region 0, see Fig. 4*a*) lies between 95% and 85% egg length, extending over 12-15 nuclei. The next most anterior region (region 1) extends around the entire circumference of the egg, being considerably broader ventrally (6-7 nuclei) than dorsally (3-4 nuclei). The remaining six regions (2-7) also extend around the whole circumference,

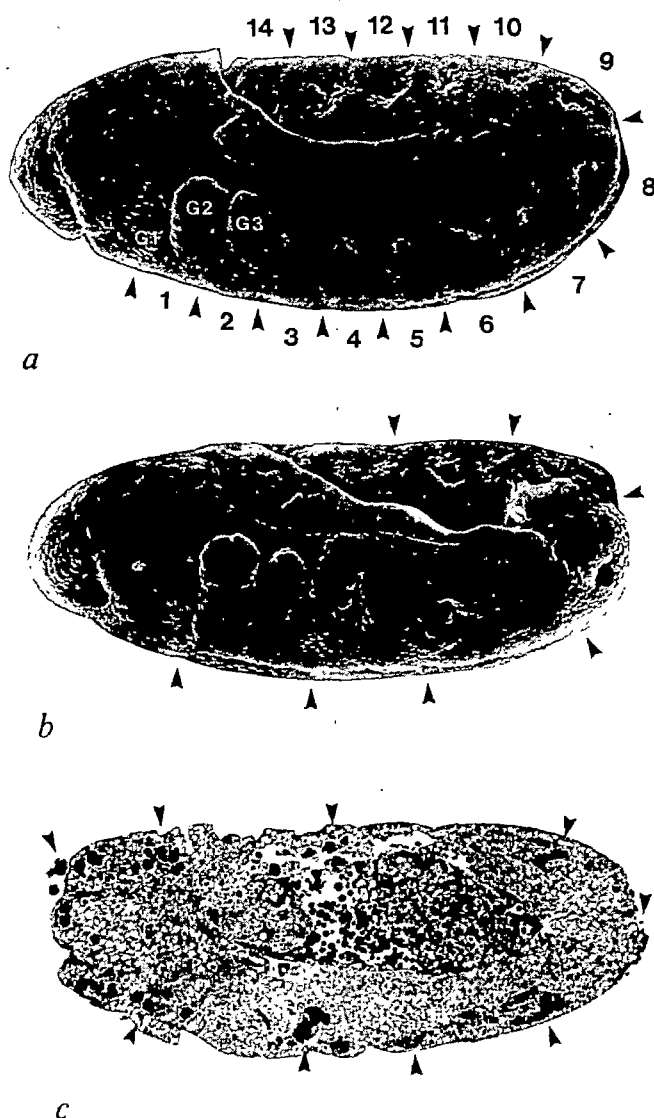


Fig. 2 In normal development, formation of the cellular blastoderm is followed almost immediately by the onset of gastrulation. Cells along the ventral midline invaginate to form the presumptive mesoderm and the ventral region of the embryo then begins to extend around the posterior pole. On completion of this process (after about 4.5 h of development at 25 °C) the embryo is folded back on itself with its posterior-most tip located dorsally just posterior to the initial position of the cephalic fold, the 'elongated germ band' stage. After a further 90 min, the first evidence of metamerism is visible⁴². The extended embryo becomes subdivided into 14 regions by superficial grooves in the ectoderm. These regions correspond to parasegments^{22,39}, the posterior compartment⁴¹ of one segment and the anterior compartment of the succeeding segment. **a**, A scanning electron micrograph of a wild-type embryo at the extended germ band stage (after about 6 h of development). Superficial grooves in the ectoderm (indicated by arrows) mark the boundaries between parasegments 1–14^{22,39}; the lobes correspond to the rudimentary gnathal appendages (G1–G3)⁴². The single large anterior-most lobe corresponds to the labrum (L). Within each of parasegments 4–13 is a deep invagination of cells which will form the tracheal system. These correspond to the position of the segment boundaries²². **b**, An embryo homozygous for the null allele¹⁸ *h*^{1.79K} at a similar developmental stage to that shown in **a**. This embryo is divided into only half the normal number of parasegments and possesses a correspondingly reduced number of tracheal pits. **c**, A lateral sagittal 2- μ m section through an embryo similar to that shown in **b**. Clusters of darkly stained dead cells are located in discrete regions along the length of the germ band (arrows). All specimens are oriented dorsal side up, anterior to the left.

and are of similar width (each 3–4 nuclei) and evenly spaced. Together, regions 1–7 extend from 75% to 20% egg length (Fig. 4d). At the same stage as the periodic pattern of *h* expression is fully resolved, transcripts from the pair-rule gene *ftz* are localized in seven stripes in the blastoderm (ref. 15 and

Fig. 3). These stripes are similar in width to those of *h* transcript at the equivalent stage.

Although some *h* transcript can be detected over the nuclei, much of the signal is located in the outer cytoplasm (Fig. 4c). This peripheral location of *h* transcripts in the cortical cytoplasm resembles that of *ftz* transcripts and contrasts with predominant localization of *Ubx* transcripts in the nucleus and inner cytoplasm²⁵.

Almost immediately after cellularization is complete, a transverse invagination of cells occurs one-third of the way from the anterior pole at 65–70% egg length¹⁹ (EL; the anterior pole is at 100% EL). This cephalic fold is the first morphological marker to appear along the antero-posterior axis of the previously uniform cellular blastoderm. The invaginating cells correspond to the primordia of the G1 and G2 segments^{19,26,27}.

At the onset of gastrulation, *h* regions 1 and 2 can be seen to flank the cephalic fold, region 1 being drawn into the anterior margin of the fold, whilst region 2 lies posterior to it (Fig. 4e). At the same stage the most anterior *ftz* region is located at the posterior of the fold (ref. 15 and Fig. 4f). As gastrulation proceeds, the periodically distributed *h* transcript rapidly decays (Fig. 3m–o). By the time the germ band has elongated, there is no evidence of the repeating pattern typical of the blastoderm. Transcript, however, is present in the hindgut and the foregut (Fig. 3p, q).

Domains of *h* and *ftz* RNA localization

To determine more precisely the relationship of the *h* and *ftz* patterns, probes for both transcripts were applied simultaneously to the same section so that the resultant signal represents the sum of the two individual signals (Fig. 5a, c). The two probes were also applied individually to the adjacent sections so that in any given embryo the signal from either *h* or *ftz* RNA alone could be distinguished (Fig. 5b, d). The mixed probes (Fig. 5a) clearly define the anterior dorsal patch of *h* RNA. Ventrally in the region between 75% and 15% EL, eight discrete regions of labelling can be resolved, each separated by a single unlabelled incipient cell. The eight labelled stripes are each about six or seven nuclei wide.

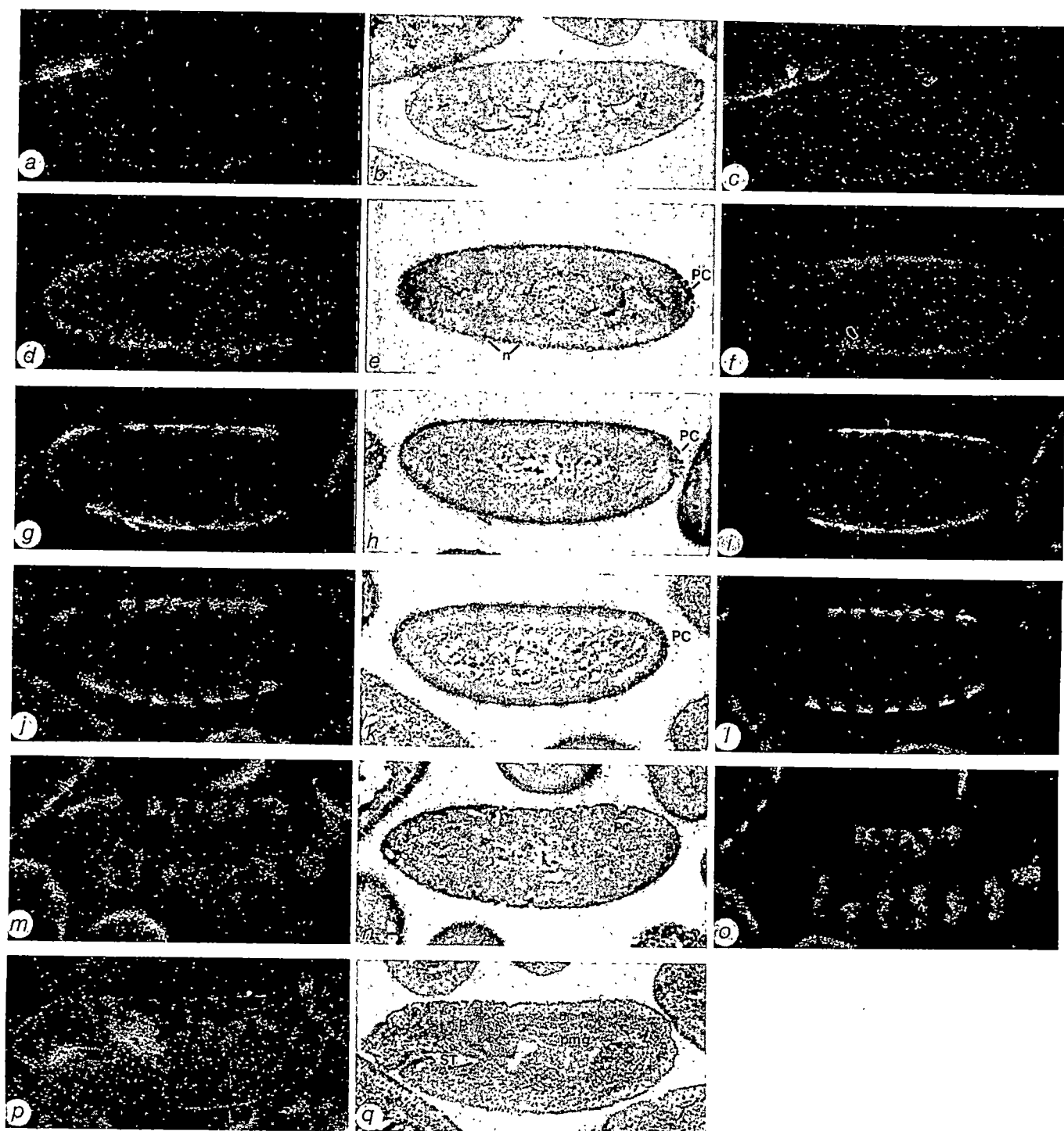
Since both *h* and *ftz* patterns consist of seven regularly spaced stripes, it is probable that the two terminal stripes in the pattern detected by the mixed probes consist of only one species of transcript. The anterior-most stripe in the mixed pattern represents *h* transcript, since *h* region 1 lies in the anterior part of the cephalic fold, whereas the anterior-most *ftz* stripe lies in the posterior part of the fold (see Fig. 4c, d). The posterior-most stripe must therefore be due to *ftz* transcript. Thus, the total domains defined by *h* and *ftz* transcripts are out of register by one metamer unit. In addition, the two patterns are out of phase with one another, though not to such a degree that they define complementary regions of the cellular blastoderm. The central six stripes must represent the addition of the two signals, with the anterior of each broad stripe being due to *ftz* signal and the posterior to *h* signal.

Maternal determinants

Polarity along the dorso-ventral and antero-posterior embryonic axes is established under the influence of maternally derived gene products¹³. The *h*-transcription pattern is asymmetrical along both these axes, implying that its generation requires such maternally acting genes. To test this possibility, *h* expression was analysed in embryos produced by two maternal-effect mutations, *dorsal* (*dl*) and *bicaudal* (*bic*).

Females homozygous for the *dl*¹ mutation produce embryos in which cellular fates are changed such that all regions of the embryo behave as though they were dorsal¹³. In the absence of the *dl*⁺ product, the *h* transcription pattern at stage 14 is altered, being approximately symmetrical along the dorso-ventral axis; this is particularly clearly illustrated at the anterior pole where transcript accumulates both dorsally and ventrally (Fig. 6b).

Homozygous mutant *bic* females produce embryos in which the anterior half is frequently replaced by a mirror-image



posterior^{13,28}. This change is invariably accompanied by a reduction in the total number of segments (see ref. 28 and Fig. 6c). Both these features are reflected in the pattern of *h* transcript distribution in stage 14 embryos derived from *bic* females. Region 0, which characterizes the anterior pole of the embryo, is frequently absent. Fewer regions of transcript accumulation are present (Fig. 6d), and their position differs from wild type.

An analogous effect on *ftz* expression has recently been reported²⁹.

Discussion

Transcription of *hairy* is first detectable during embryogenesis at the syncytial blastoderm stage. The transcripts, which are initially distributed throughout the embryo, become localized periodically along its antero-posterior axis at about the time the

body plan is thought to be established. This localization is presumably intrinsic to the process of segmentation because absence of *h*⁺ activity during embryogenesis causes a similarly periodic pattern of deletions along this axis. These deletions arise following a failure of the subdivision of the early embryo into the appropriate number of metameric units and are associated with localized cell death.

There are eight regions of *h* transcript accumulation in the cellularizing blastoderm, equivalent to the number of regions deleted from the larval cuticle. Superposition of the pattern of hybridization and the blastoderm fate map (Fig. 4d) suggests that these sites correspond broadly to the locations of the primordia of the missing structures. Similarly, *ftz* transcripts are localized in seven regions which correspond approximately to the primordia of structures deleted in *ftz* mutant larvae¹⁵.

Fig. 3 (Left) Comparison of *h* and *ftz* transcription during early embryogenesis. The central panels are bright-field images of sections through successively older embryos. In all cases, anterior is to the left. All sections are sagittal (dorsal side up) except for those in *d-f*. After nine rapid divisions in the yolkly central region of the egg, most nuclei migrate to the peripheral cytoplasm to form the syncytial blastoderm^{24,43}. Here they divide a further four times, before cell membranes begin to grow inwards, isolating each nucleus and forming the cellular blastoderm (stage 14 of Foe and Alberts²⁴). Stages were determined by comparing the density of nuclei along the egg periphery. The pattern of *h* or *ftz* expression in each embryo is revealed by the dark-field images of adjacent sections hybridized with ³⁵S-labelled *h* probe (left) or ³H-labelled *ftz* probe (right) respectively. *a-c*, Stage 10 embryo; no expression of either *h* or *ftz* has been detected at this stage. Exposure, 30 days. *d-f*, Frontal sections of stage 13 embryo. Expression of *h* extends around most of the circumference of the embryo with the possible exception of the posterior polar region. In contrast, *ftz* expression is limited from the outset to a region from between 65% to 15% EL. In both cases the silver grains are most densely localized over the nuclei (n); the intensity of nuclear labelling is not uniform. PC, pole cells. Exposure, 30 days. The three embryos shown in the next nine panels are directly comparable being hybridized under precisely the same conditions. Exposure, 20 days. *g-i*, Early stage 14 embryo. Anteriorly, the *h* transcripts have become restricted to the dorsal surface. The remaining region is becoming discontinuous, with seven regions being apparent dorsally. At the same stage, the *ftz* transcripts are also starting to become localized. By mid-stage 14 (*j-l*), both *h* and *ftz* transcript patterns are well resolved into a series of stripes along the antero-posterior axis. This pattern begins to decay with the onset of gastrulation (*m-o*), the decay appearing more rapid in the case of *h* (compare the signal levels in *m* and *o* with the neighbouring stage 14 blastoderm in the top left corner). Note, however, that the anterior dorsal patch of *h* transcript remains prominent at this stage. By the completion of germ-band extension the periodic pattern of both transcripts has decayed. Expression of *h* is now detectable in the developing hindgut and foregut (the proctodaeal (P) and stomodaeal (ST) invaginations respectively) but not in the posterior midgut (pmg), and persists in this region throughout the elongated germ-band stage. *p, q* Show such an embryo just prior to the onset of germ-band shortening. Exposure, 11 days.

Methods. Eggs were collected from rapidly laying fly populations on yeast/agar medium and incubated at 25 °C until the desired developmental stage had been reached. Prior to fixing, the chorion was removed by incubation in 14% sodium hypochlorite. Fixation was in 4% paraformaldehyde in phosphate-buffered saline. The embryos were first pre-fixed by the phase partition method⁴⁴; vitelline membranes were then removed by the method of Mitchison and Sedat⁴⁵. After rehydration, embryos were fixed for a further 15 min, dehydrated and embedded in paraffin wax. Sections (6 µm) were cut and mounted on glass microscope slides, coated with poly-D-lysine. After removal of the wax with xylene, sections were rehydrated and prepared for hybridization essentially as described elsewhere^{46,47} except that the slides were additionally treated with 0.25% acetic anhydride in 0.1 M triethanolamine pH 8.0 for 10 min at room temperature before the final dehydration⁴⁸. DNA templates for transcription were subcloned into pSP64 or pSP65⁴⁹ and ³H- or ³⁵S-labelled RNA synthesized in a 20-µl reaction containing 40 mM Tris-HCl pH 8.0, 6.0 mM MgCl₂, 10 mM dithiothreitol, 4 mM spermidine, 100 µg ml⁻¹ bovine serum albumin, 0.1% Triton X-100, 1 U µl⁻¹ RNasin (Amersham), 100 µg ml⁻¹ linearized template DNA, 500 µM ATP and CTP with 100 µM ³H-GTP and NTP (NEN) or 500 µM ATP, GTP and CTP with 10 µM ³⁵S-UTP (NEN) and 0.2 U µl⁻¹ SP6 polymerase (Anglian Biotechnology) incubated at 30 °C for 4 h. These reaction conditions were found to maximize the yield of full-length transcripts (K.R.H., unpublished observations). The reaction was terminated by the addition of DNase I to 5 µg ml⁻¹ followed by incubation at 37 °C for 30 min. Probes were size reduced to an average of 50-100 bases using alkaline hydrolysis at pH 10.4 for 40 min at 60 °C⁵⁰. Final specific activities were 1.1 × 10⁸ d.p.m. µg⁻¹ for the ³H-labelled probes and 1.2 × 10⁷ d.p.m. µg⁻¹ for those labelled with ³⁵S-thiophosphate. The templates have been described previously^{21,51}. These probes were used at a final concentration of ~0.3 ng per µl per kb in 50% formamide, 10% dextran sulphate, 0.3 M NaCl, 10 mM Tris-Cl, 10 mM NaPO₄ pH 6.8, 5 mM EDTA, 1 × Denhardt's, 10 mM dithiothreitol, 1 mg ml⁻¹ transfer RNA⁵⁰. A 20-µl aliquot of this solution was dispensed on each slide and spread over the sections with a siliconized glass coverslip. Slides were sealed in plastic boxes saturated with wash buffer (50% formamide, 0.3 M NaCl, 10 mM Tris-HCl, 10 mM NaPO₄ pH 6.8, 5 mM EDTA, 1 × Denhardt's) and incubated overnight at 50 °C. Coverslips were removed by immersing slides in wash buffer plus 10 mM dithiothreitol (WDTT). Slides were washed in fresh WDTT for 4-5 h at 50 °C and treated with RNase A at 20 µg ml⁻¹ in 0.5 M NaCl, 10 mM Tris-HCl pH 8.0, 1 mM EDTA (NTE) for 30 min at 37 °C⁵⁰. Subsequently, they were washed in NTE for 1 h before a further final overnight incubation in WDTT at 50 °C. After dehydration of the sections, slides were coated with Kodak NTB2 emulsion, dried, stored at 4 °C and developed with Kodak D-19 developer (1:1 dilution) for 2 min at 20 °C after the stated exposure time. Controls using sense-strand hybridizations and treatment of antisense-strand hybridizations with RNase A in low salt (data not shown) indicate that the signals are due to specific hybridization.

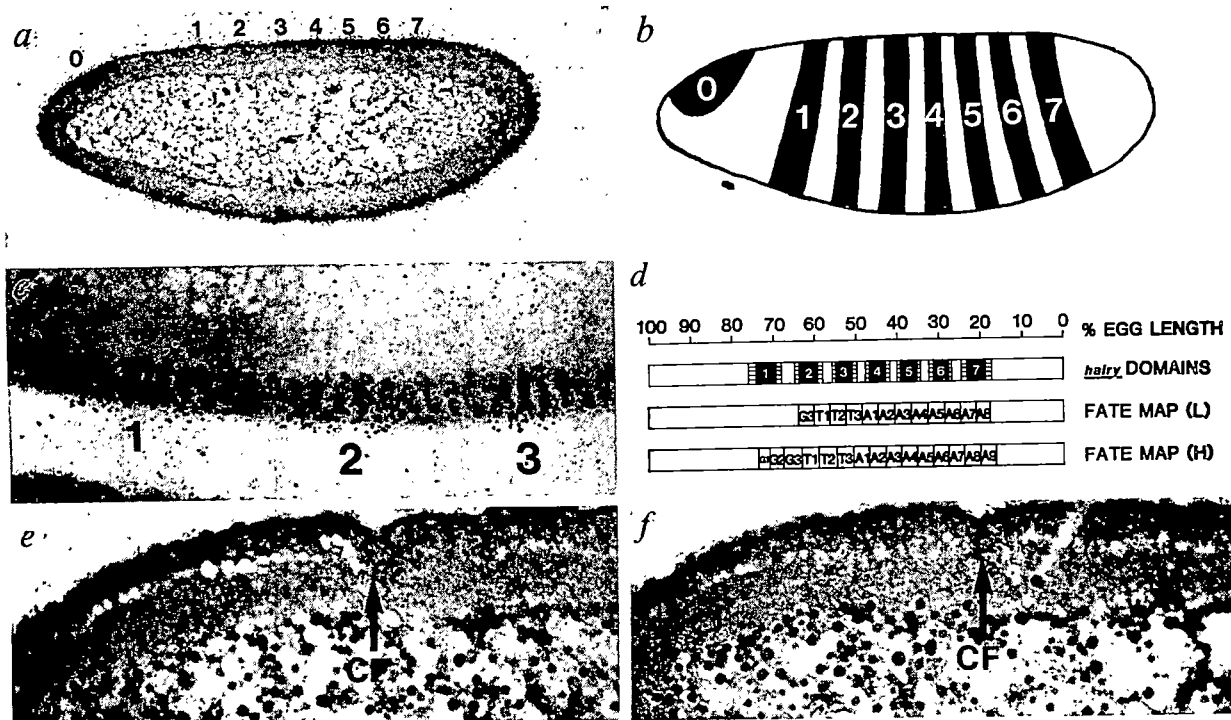


Fig. 4 *a*, Medial sagittal section of a mid-stage 14 blastoderm hybridized with ³⁵S-labelled *h* probe, revealing the eight regions (designated 0-7) of *h* transcript accumulation. *b*, A projection, onto the medial sagittal plane, showing the asymmetry of the pattern of *h* expression. This is based on several partial serial reconstructions. *c*, Detail of the ventral side of a late stage 14 blastoderm hybridized with ³H-labelled *h* probe. Note that domain 1 is broader than domains 2 or 3 which are both 3-4 cells wide. *d*, Projection of the regions of *h* RNA accumulation onto blastoderm fate maps (Lohs-Schardin *et al.*¹⁹ (L) and Hartenstein *et al.*²⁶ (H)). The filled regions show the average localization which involved medial frontal sections of six separate embryos (hatched regions indicate the s.d. of these measurements). Note the approximate correspondence with the primordia for regions deleted in the *h* mutant phenotype (compare with Fig. 1*a*). The two fate maps differ from one another in the exact location of the primordia. Although their estimates for the average size of the primordia (3.9% and 3.65%) are lower than ours (4.2 ± 0.16%), this is due mainly to the broadening of the *h* pattern seen anteriorly. Detailed comparisons with either fate map are not warranted since slightly different assumptions⁵² or level on the dorso-ventral axis¹⁹ were used to construct these maps. *e, f*, *In situ* hybridizations with *h* and *ftz* probes respectively to serial sections of the same early gastrula, illustrating the relationship of the regions of hybridization to the cephalic fold (CF).

One interpretation of these findings is that *h* or *ftz* are autonomously required for the specification of alternating sets of blastoderm cells; mutations in one or other gene would accordingly result in the death of these cells or their descendants. Since the *ftz* and *h* deletion patterns are approximately complementary, a *prima facie* expectation might be that the patterns of expression would show an equivalent relationship. Our results demonstrate, however, that even before cellularization is complete, some incipient cells in the metameric region of the blastoderm express neither gene at significant levels. Such cells could require the expression of one or more of the other pair-rule genes for their specification (see refs 15, 30).

We emphasize, however, that approximate correspondence between the sites of RNA localization and the primordia of structures deleted in the absence of *h* or *ftz* expression does not necessarily imply an autonomous requirement for either gene in these cells. An alternative possibility is that the pair-rule genes are a functionally heterogeneous class, the various genes acting in different ways to establish the metameric pattern.

Although the initiation and subsequent spatial distribution of *h* and *ftz* transcription follow a similar time course, they do exhibit some notable differences. Whereas *h* is initially expressed around most of the egg circumference, expression of *ftz* is restricted from the outset within the presumptive post-oral metameric region of the embryo. Moreover, the disposition of the *h* transcriptional regions appears less uniform than those of *ftz*; regions 0 and 1 are somewhat broader and region 4 slightly weaker than the remaining regions. These differences suggest that the two genes function at different levels.

The striped pattern of both *ftz* and *h* is unstable and has decayed within an hour of the cellular blastoderm stage, the decay of *h* appearing more rapid than that of *ftz*. This latter finding argues against a role for the pair-rule genes in maintaining the segmental pattern. Although it is conceivable that long-lasting protein products of these genes could effect such a function, it is more likely that they act as transient signals which regulate the expression of other genes. A primary candidate for such a gene is *engrailed* (*en*), which is first expressed in a periodic pattern in the cellular blastoderm^{31,32}. In contrast to both *h* and *ftz*, *en* expression continues throughout development in this spatially restricted pattern, reflecting its role in directing the development of the posterior compartment of each segment³³⁻³⁵.

The finding that *h* transcripts accumulate in the anterior region of the embryo throws some light on the question of head segmentation: region 0 includes the location of the labral primordium which is similarly restricted to the dorsal surface of the embryo. Although the lobed appearance of the labrum in the extended germ band (see Fig. 2a) is suggestive of an appendage and hence of its being segmental in origin, such an interpretation has previously been questioned on other morphological grounds³⁶. This would leave only one segment anterior to the mandibular (G1) segment, namely the antennal, the primordium of which maps between the *h* regions 0 and 1. Recently, however, evidence from fate mapping studies^{20,37} together with the analysis of homoeotically transformed larvae³⁸ has suggested the presence of at least two segments anterior to G1. In addition, *in situ* analysis of *engrailed* transcription clearly shows it to be expressed in two regions anterior to G1, one of which is located in the labrum^{3,39}. Taken together, these findings suggest a metameric origin for the labrum, with expression of *h* being required for its establishment.

The significance of the presence of *h* transcripts in the presumptive fore- and hindguts of the extended germ band is at present obscure. We note, however, that *en* is also expressed in these cells at the same stage^{31,32,39}.

The pattern of *h* RNA is not axially symmetrical, the stripes being broader ventrally than dorsally and inclined to the embryonic axis (see Fig. 4b). Presumably this reflects the true shape of the fate map at this stage, since such a pattern is seen well before the beginning of the movements of gastrulation. This contrasts with the assumption of a simple perpendicular

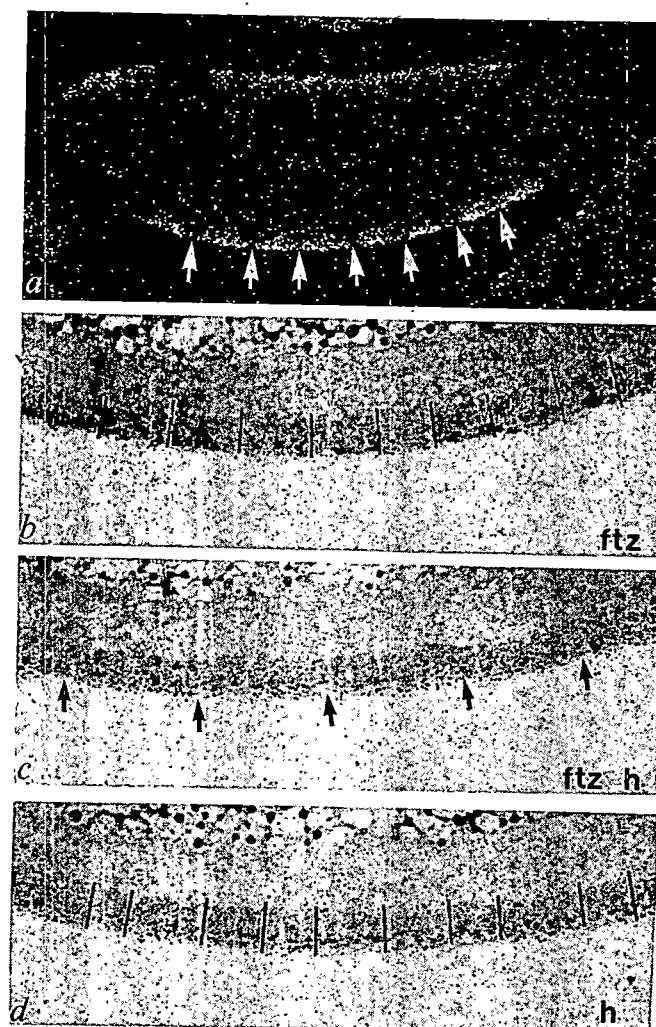


Fig. 5 The spatial relationship of *h* and *ftz* domains. The specific activities of the *h* and *ftz* probes were controlled such that each probe produced a similar signal intensity after a given exposure. Mixtures of the two probes, in equal concentrations, were applied to various sections of wild-type embryos. Adjacent sections of these embryos were hybridized with either the *ftz* or *h* probe alone. **a**, Dark-field image of a sagittal section of a stage 14 embryo hybridized with both *ftz* and *h* probes. Note the dorsal anterior patch of labelling, typical of *h* domain 0. The ventral surface shows a pattern of labelling which is discontinuous, the boundaries between the regions of highest labelling being indicated by the arrows. These subdivide the region into eight. Dorsally, the pattern of labelling is more uniform. Note that the posterior-most *ftz* stripe is always broader than the remainder (see, for example, Fig. 3c). **b**, **c**, **d**, Bright-field images of the ventral regions of successive sections of the embryo shown in **a**, hybridized with *ftz*, *ftz* and *h*, and *h* probes respectively. The labelled region in each case is indicated by black lines. A given cell is taken to be labelled if two or more silver grains are present in its outer cytoplasm. In **c**, arrows indicate cells adjudged to be unlabelled. The size of the labelled domains in **c** is consistent with their being due to the superposition of the individual *h* and *ftz* domains, the *ftz* domain being two nuclei anterior to the *h* domain. Note that the width of the unlabelled regions in the embryo hybridized with *ftz* never exceeds that of the regions labelled with the *h* probe.

relationship between the segmental primordia and the major axis of the blastoderm made in the fate-mapping study of Hartenstein *et al.*²⁶, but confirms the earlier observations of Lohs-Schardin *et al.*¹⁹. This asymmetry reflects a requirement for determinants of both major embryonic axes in the establishment of the *h* pattern. Our results show that in the absence of dorso-ventral polarity the *h* transcription pattern becomes approximately symmetrical. This could imply the existence of a cartesian

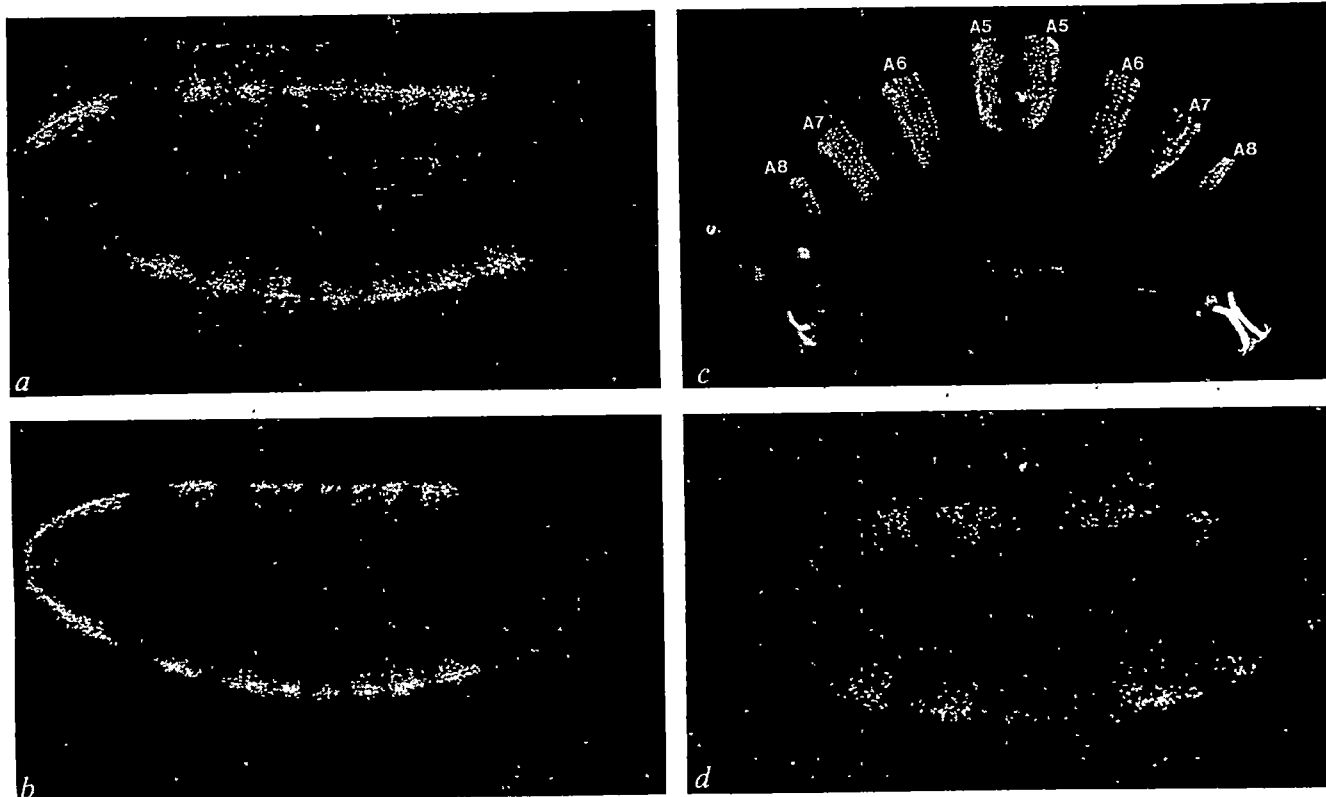


Fig. 6 *a*, Dark-field image of section shown in Fig. 4*a*, illustrating the wild-type *h* pattern. *b*, Sagittal section of a mid-stage 14 blastoderm derived from a *dl¹/dl¹* female hybridized with *h* probe. Note the accumulation of *h* transcript both dorsally and ventrally at the anterior pole. *c*, Ventral cuticular pattern of a pharate first-instar larva derived from a homozygous *bic^{M241}* female. The anterior region is replaced by segments of posterior character (A5–A8) in mirror image to the normal posterior. Note the reduction in the overall number of segments. This phenotype is variable but fully penetrant at 18 °C. *d*, Parasagittal section of a sibling of the animal shown in *c* fixed at mid-stage 14 and hybridized with *h* probe. The labelling pattern reveals a reduction in the number of regions of *h* transcript accumulation. This pattern can be interpreted as representing two regions, 6 and 7, in mirror-image symmetry, consistent with the reduction in segment number typical of these embryos (compare with Figs 1*a* and 4*a*). Note also the absence of the anterior dorsal region (0) of transcript accumulation (compared with *a* and *b*).

coordinate system, *h* responding to different antero-posterior values according to position along the dorso-ventral axis. Alternatively, the coordinate system may itself be distorted, reflecting directly the shape of the blastoderm fate map.

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Are some BL Lac objects artefacts of gravitational lensing?

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BL Lac objects are extragalactic, highly variable, polarized sources with significant emission from radio to X-ray wavelengths. However, despite extensive research, their nature and origin remain unclear. We propose here that a significant fraction of the BL Lac objects are optically violently variable quasars (OVVs) whose continuum emission has been greatly amplified, relative to the line emission, by point-like gravitational lenses (either stars or black holes) in intervening galaxies. Several anomalous physical and statistical properties of BL Lacs can be understood on the basis of this model, which is immediately testable on the basis of absorption line studies and by direct imaging.

The class properties of BL Lacs are fairly well-defined, although this may mask an underlying heterogeneity which will retard our comprehension as it did previously with Seyferts, nebulae, or even stars. They are typically point-like (<2 arc s) with the occasional case of extended optical or radio emission perhaps critical to understanding them. In all characteristics so far described, they resemble the OVVs, a class of quasars known for high polarization and variability. They also resemble the OVVs in their optical continuum spectra, where both are steeper than typical quasars, and in their ratio of radio-to-optical where both show higher values. However, they are distinct from the OVVs and from other QSOs in the absence or near-absence of the normal QSO emission lines, even though, during low periods of continuum, emission lines are occasionally seen¹. Statistically, BL Lac objects are uncommon among the brighter extragalactic point sources, constituting perhaps 10% of all objects brighter than $m_B = 16$ (~ 35 objects over the whole sky), and even less common at fainter magnitudes². This is also true of their X-ray properties, where the number-magnitude relation flattens out much earlier than it does for normal QSOs³.

The conventional interpretation is that BL Lac objects are one type of active galactic nuclei. This is supported by the fact that several have been found in relatively small-redshift elliptical galaxies⁴. The variability, high polarization and absence of extended radio structure are attributed to relativistic beaming⁵. While few detailed models have been constructed⁶, the picture is moderately persuasive and is likely to be correct for some subset of the BL Lac objects. There are, however, seriously anomalous features of the observations which are inconsistent with or unexplained by the standard interpretation.

First, there are physical problems. The emitted ionizing continuum would be expected to be absorbed by ambient gas in the elliptical galaxy, with some radiation ultimately degraded to the $H\alpha$ line. Using standard results⁷, we find that if the gas in the elliptical satisfies the inequality $n_e M_{\text{gas}} > 10^{8.5} M_\odot \times \text{atoms cm}^{-3}$, then $H\alpha$ would be detectable in a $m_B = 16$ BL Lac at $z = 0.05$. This is comparable with the amount of gas found in ellipticals, and so, if the gas has not been ejected due to violent nuclear activity, might be expected to be seen in some cases.

Then come the problems with the popular beaming model: it is unlikely that it can, alone, account for the properties of compact radio sources. If the sources whose beams are pointed away from our line-of-sight are identified with radio-quiet quasars, which are otherwise identical to compact sources, why do the compact sources have isotropically emitting haloes, while the extended sources do not? If, on the other hand, extended steep spectrum sources are to be identified with the sources beamed away from us, then the statistics fail, in the sense that there are too few extended QSOs to be consistent with a random-pointing beam. Also, there is at least one extended source

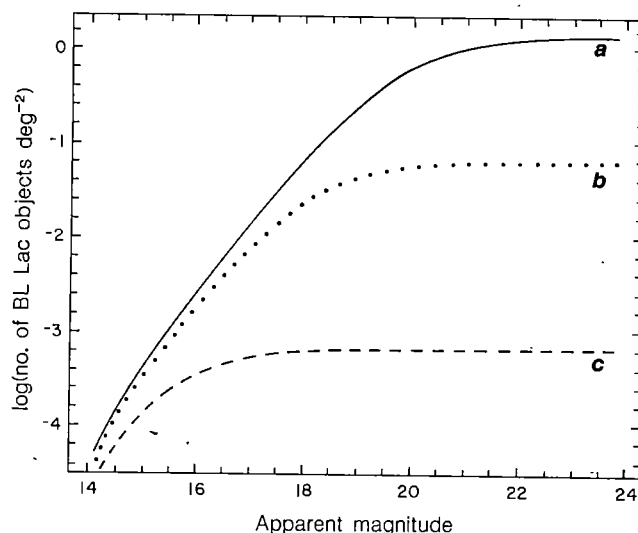


Fig. 1 Number-magnitude relation for OVVs that have been amplified by mini-lensing by at least a factor of 2(a), 10(b) or 100(c), per unit square degree. The OVVs are supposed to have the same luminosity function as the QSOs¹⁶, and to constitute only a fraction $f = 10\%$ of all QSOs.

(3C179) that shows superluminal expansion, and one compact source (4C39.75) that does not.

There are also statistical incongruities. If BL Lacs are nearby objects, a euclidian number-flux relation in the X-ray $N \propto f_X^{-1.5}$ would be expected which is significantly steeper than observed³ for $f_X < 10^{-12} \text{ erg s}^{-1} \text{ cm}^{-2}$. This result is not biased by the usual incompleteness of the optical data. Then there is the distribution in distance. Only a small number of BL Lac objects have been examined for absorption line redshifts, with identified systems ranging from $z_{\text{abs}} = 0.424$ (PKS0753+178)⁸ to $z_{\text{abs}} = 1.719$ (0215+15)⁹. This is not unlike the QSO distribution, but it makes anomalous the relatively large number of objects apparently seen in nearby ($z < 0.2$) bright ellipticals. If the typical QSO has $z = 1-1.5$, then the nearest of a sample of 60 should have a $z = 0.16$ (for $\Omega = 1$). This argument is valid for QSOs where the nearest of the hundred brightest objects is 3C273 with $z = 0.158$. But it is highly improbable ($< 10^{-2}$ to find a single object) to have a fraction $\sim 50\%$ of the objects with known redshifts such that $z < 0.2$ as is apparently the case for the BL Lacs. One could avoid this dilemma by imagining the number-luminosity relation to be very steep with many more intrinsically faint than bright objects in comparison with normal quasars, but then the relatively flat number-magnitude relation² becomes even more peculiar. But, if we treat the nearby BL Lacs apparently in galaxies as statistical accidents to avoid these difficulties, we are faced with a wild improbability: one quasar¹⁰ out of 2,000 has been found with its image superimposed on the central regions of a galaxy having $z < 0.2$; so 11 of the 50-odd BL Lacs is extremely unlikely on statistical grounds.

On the basis of this argument, it is attractive to look for an explanation for the phenomenon in which the BL Lacs are at cosmological distances, but the apparently foreground galaxies, whether seen in absorption or in emission, are not there accidentally. We propose that a significant fraction have been gravitationally lensed by point-like objects in the foreground galaxies. Typical optical depths for mini-lensing¹¹ are of the order of 10% for $\Omega_1 = 0.2$, if the haloes of galaxies are made of stellar-like objects. Thus we propose that the OVVs are intrinsically small (or 'beamed') objects, on the basis of the usual variability arguments, and that of the order of 10% of them have been magnified by mini-lenses in intervening galaxies to present us with a BL Lac object. This is made possible by the differing sizes of the continuum-emitting regions ($r \sim 10^{15} \text{ cm}$) and of the line-emitting regions ($r \sim 0.1-1 \text{ pc}$). The line-emitting regions are too large to be lensed by stars, or even black holes up to

the mass of 10^1 – $10^3 M_\odot$ (ref. 12). This model is similar to that proposed by Barnothy and Barnothy¹³, but we are applying it here to only a small subset of QSOs. Normal QSOs are too large, in general, to be affected by mini-lensing.

In recent studies of the effects of lensing on the statistics of QSOs^{14,15}, we found that a class of objects with the postulated properties should be expected to exist on statistical grounds. We have computed the number-magnitude relation for all OVVs that have been brightened by mini-lensing by at least a factor of 2, 10 or 100; the adopted luminosity function is that given by Schmidt and Green¹⁶, truncated at $z=3.5$, and scaled down by a factor of 10, to account roughly for the incidence of OVVs among regular quasars. The results are shown in Fig. 1. Given the prominence of the emission lines of QSOs, it is likely that only objects whose continuum has been brightened by at least a factor of 10 would be classified as BL Lacs. Figure 1 shows the expected apparent magnitude distribution for such objects, assuming a density of point-like lenses equivalent to $\Omega_l=0.2$ (refs 14, 15). The striking feature is the high apparent luminosity at which the counts flatten: $m_B=16$ –18. This agrees qualitatively with the X-ray observations, and explains the negative results of optical searches for faint ($m_B \sim 20$) polarized objects²: both surveys investigated only a small area of sky, where but a handful of objects would be found on the basis of the statistics of regular quasars. On the contrary, we predict that searches would be most successful for large areas of sky and relatively bright limiting magnitudes. Our computation predicts ~ 100 BL Lacs over the whole sky, in agreement with the 50-odd objects discovered in (mostly) the Northern Hemisphere.

The redshift distribution of BL Lacs would be, of course, an insurmountable problem for our model if we accepted that the redshifts attributed to them are their own. But in our model it is natural to suppose that the stellar spectra seen in either emission or absorption are due to lensing galaxies, which are not physically associated with the BL Lacs; the latter are instead at cosmological distances. The redshift distribution expected for BL Lacs, for limiting magnitude of 16, is shown in Fig. 2; it mimics that of QSOs quite closely¹⁶.

There remains an apparent discrepancy for this picture in the comparison between predicted and observed redshift distributions for the lensing galaxies, which lie relatively close to us. Models¹¹ (especially Fig. 2 of ref. 11) show that they should primarily have $z \sim 0.2$ – 0.7 , if they are uniformly distributed in space ($n_l = n_0(1+z)^3$), while we know of at least eight objects¹⁷ which show absorption lines due to stellar spectra, all with $z < 0.2$. But this problem arises also when one considers normal QSOs and lensing by a galaxy, as opposed to mini-lenses; of the six known lensed quasars, one¹⁰ is lensed by a low-redshift galaxy. A tentative explanation for this may be offered (B. Paczynski, personal communication). In accounting for the large splitting and amplification of the known multiple quasars, it was pointed out¹¹ that, if the galaxies are in a cluster with surface mass density Σ approaching the critical surface mass density Σ_{crit} , then the flux is increased by $(1 - \Sigma/\Sigma_{crit})^{-2}$. Similarly, although the analogy is imperfect because the mass distribution in the lensing galaxy will be very grainy, we may expect mini-lensing to be most effective if the stars are located in a galaxy having a mean surface mass density close to the critical density. We find (equation 2.35 of ref. 11) that $\Sigma_{crit} = H_0 c / (4\pi z_l G) = 0.12 h / z_l \text{ g cm}^{-2}$, for $z_l \ll z_{QSO}$, regardless of cosmology. But the mean surface density within the de Vaucouleurs radius is relatively independent of luminosity for elliptical galaxies and is typically of the order of $(1-2)h \text{ g cm}^{-2}$ (ref. 18). Thus, normal ellipticals are near the critical surface mass density for redshifts of the order of $z \sim 0.05$ – 0.1 , which will make mini-lensing in this redshift range more prominent due to the 'amplification bias' already noted¹¹. As the lensing properties approach those for continuum mass distributions as $\Sigma \rightarrow \Sigma_{crit}$, we would expect some BL Lacs in this redshift range to show multiple images, as is the case for the object observed by Huchra *et al.*¹⁰.

Monte Carlo simulations of flux variations due to mini-lensing¹⁹ for 0957+561B show that an average amplification

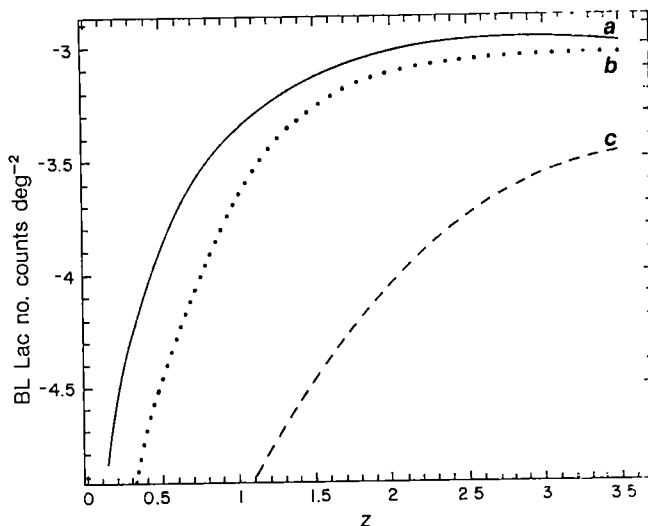


Fig. 2 Redshift distribution for all OVVs with $m_B \leq 16$, which mini-lensing has amplified by at least a factor of 2(a), 10(b) or 100(c), per unit redshift.

$A \sim 8$ can be obtained for a sufficiently small quasar; the time-scale for flux variation is ~ 5 yr for solar mass stars, a value not inconsistent with that of the observed large-amplitude optical fluctuations. Note also that the relatively small amplitude of the fluctuations obtained by Young¹⁹ (a factor of ~ 2) may be due to the large beam size assumed for 0957+561, which is not an OVV. Also, large swings in the polarization vector during outbursts may be due to mini-lensing²⁰: when the optical depth to mini-lensing is $\tau_{ml} \sim 1$, any subregion of the quasar image can be amplified; which region actually is, depends on the very non-local cooperation of all mini-lenses. If the polarization changes intrinsically in the source, the star motions cause differently polarized regions to be amplified at different times, and the polarization vector to swing during outbursts.

We emphasize that mini-lensing is not alternative to beaming, but depends on it, in that mini-lensing can cause some beamed sources (OVVs) to become BL Lacs. In fact, mini-lensing can relieve the statistical problems of the beaming hypothesis. Tests comparing local densities of BL Lacs and of QSOs have concluded¹⁷ that it is difficult to find a class of radio sources which is numerous enough to be consistent with the statistics of a randomly pointing small beam. If our hypothesis is correct, though, the BL Lacs are at cosmological distances, and this implies that the local density of BL Lacs has been overestimated¹⁷.

The last objection to the mini-lenses hypothesis that we consider here is the following: because we assume that mini-lensing can amplify the optical continuum, but not the line-emitting region, the radio continuum ($r \sim 1$ pc) should not be amplified, and OVVs and BL Lacs should have significantly different (by a factor of 10) radio-to-optical ratios. This objection neglects K-corrections, which are necessary in our hypothesis because the BL Lacs are at cosmological distances. The radio continuum is rather flat ($\alpha_r \sim 0$), while the optical continuum is steep ($\alpha_{opt} \sim 2$), so neglect of K-corrections (of order $(1+z)^2 \sim 10$ for cosmological redshifts) can easily mask different radio/optical flux ratios.

Our simple model has several consequences which make it easy to test.

- (1) One should expect to see absorption redshift systems in a significant fraction of BL Lacs, as they lie at cosmological distances, especially in the objects that show absorption due to stellar spectra. In these objects, one expects to have $z_{abs} > z_{gal}$. As it has been argued that most quasars have $z \leq 2$, we also expect this to be the case for BL Lacs, as observed²¹.
- (2) We predict a continuity of properties between BL Lacs and OVVs, mostly in the relative importance of the line and of the

continuum emission. This continuity has been noticed already for several other properties (polarization, fluctuations of flux and polarization and their timescales)²², with the BL Lacs always having the most extreme properties.

(3) The position of the optical continuum source should not be centred on the image of the associated galaxy, where observed. Two examples of this sort^{1,23}, where the distances are 6.5 and 12 arc s, respectively, are known already, whereas if the BL Lac object is in the galaxy, it should always be centrally located. One might also expect a few objects to have multiple images, or the radio source not to coincide with the optical galaxy²⁴.

(4) But if, contrary to our hypothesis, the strong hard spectrum source sits in the core of a nearby elliptical galaxy, one would often expect to see Balmer line emission from the galaxy, while in our model this should not happen, as there is no physical connection between the hard spectrum source and the galaxy.

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GB841215, the fastest γ -ray burst?

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In the 12 yr since the discovery of γ -ray bursts by Klebesadel *et al.*¹, several hundred of these enigmatic events have been observed and catalogued (see, for example, refs 2–5). Their time histories have exhibited a tremendous diversity: they can have durations of milliseconds or minutes; they may contain one or a dozen individual peaks; and they can be highly impulsive or slowly varying. Possibly the only truly unifying observational property of γ -ray bursts is that, with very few exceptions^{6,7}, the bulk of the energy output seems to be in the form of γ -rays. Here we report the detection on 15 December 1984 at 08.25 UT of an extraordinary outburst, qualitatively different in appearance from all previously observed γ -ray bursts. It is described most conveniently as a 'classical' multi-peaked, hard-spectrum burst that has been compressed in time by a factor of 10–100 while simultaneously having its intensity increased by a like factor (thus conserving fluence). Its peak intensity was much higher than any other known γ -ray burst except

for GB790305b, which had an unusually soft spectrum and other unique features that set it apart from 'classical' γ -ray bursts⁸. However, a key point is that if the intensity of GB841215 had been 'normal', its narrow individual spikes would not have been statistically significant with current instrumentation, and the event would have had the appearance of a rather ordinary, short γ -ray burst.

GB841215 was observed by the Los Alamos γ -Burst Detector experiment on the Pioneer Venus Orbiter (PVO)⁹, by the UCB/Los Alamos Solar X-ray/ γ -Ray Burst experiment on the International Cometary Explorer (ICE, previously ISEE 3)¹⁰, and by the medium-energy detectors¹¹ on the European Space Agency's X-ray observatory EXOSAT. Figure 1 shows the time history of GB841215 as obtained by the ICE and PVO experiments. Due to excessively high counting rates induced by this event, EXOSAT suffered a 'crash' of its onboard computer and lost all useful data except for an approximate event onset time. The time bins in Fig. 1 are of unequal length because both the ICE and PVO experiments store data in memories that automatically convert to a 'time-to-spill' mode whenever the rate exceeds 1,365 s⁻¹, that is, the higher rates are derived from the time interval required to accumulate a specific number of counts (16 in Fig. 1a and 32 in Fig. 1b). The statistical properties of such a plot are quite different from those of the familiar Poisson distribution and tend to produce a spikier appearance because the fractional standard deviations do not decrease with increasing rate. The rate changes seen in Fig. 1a, b are significant if they exceed a factor of ~ 2 or ~ 1.4 , respectively. Thus, during the ~ 0.3 -s impulsive phase of the event, at least seven distinct peaks are evident in both parts of Fig. 1, with the sharpest of these having a full width of ≤ 0.005 s in Fig. 1a. No other γ -ray burst has exhibited such rapid multi-peaked structure (hence the word 'fastest' in the title, even though GB790305b may have had a faster rise time). However, it would be unwarranted to conclude that such narrow multiple spikes are rare or unique, because the ability to detect rapid variations is a strong function of intensity—especially in systems with rate-dependent time resolution. If GB841215 had been a factor of 10 or more weaker, it would have had peak rates comparable with other intense bursts, but, its remarkable signature would not have been discernible. Instead, a rather uninspiring 0.3-s event with probable ~ 0.1 -s structure would have been observed. (In fact, other short events observed by PVO have had marginally significant—but never convincing—indications of very fast structure.)

Because of the different energy thresholds of the two experiments (260-keV minimum for the ICE memory data versus 100 keV for PVO), the respective time histories are qualitatively different. In Fig. 1a (higher threshold), the peaks are sharper and the 'valleys' are broader and deeper, relative to b. Evident only in Fig. 1b is the beginning of a weak tail with a softer spectrum that is present for ~ 10 s. The tail does not show any clear modulations, but we have not yet thoroughly investigated this matter. The spectrum of GB841215 is generally very hard, with considerable emission above 1.5 MeV. The total >30 -keV fluence of 4×10^{-4} erg cm⁻² is large, but not exceptional. The peak intensity of $\sim 2.5 \times 10^{-3}$ erg cm⁻² s⁻¹ is exceptional, and has been exceeded only by GB790305b.

The location information for GB841215 is derived from only a three-satellite arrival-time analysis. Accordingly, there are two non-redundantly determined error boxes located approximately symmetrically with respect to the ecliptic plane. (We are still searching for additional data that could lead to a better localization for this event, but the probability of success is not high.) The $\geq 90\%$ confidence error boxes are centred at $(\alpha, \delta)_{1950} = (16\text{ h } 44\text{ min } 48.2\text{ s}, -6^{\circ}42'59'')$ and $(16\text{ h } 17\text{ min } 09.2\text{ s}, -42^{\circ}24'10'')$, or $(L_2, B_2) = (11.2, 23.6)$ and $(338.8, 5.3)$. They are best described as 2° -long segments of an annulus whose axis is located at $(\alpha, \delta)_{1950} = (21\text{ h } 13\text{ min } 19.4\text{ s}, -18^{\circ}10'20'')$ and whose radius is $66^{\circ}13'19'' \pm 4''$. The ends of the two segments are located at $(16\text{ h } 43\text{ min } 37.9\text{ s}, -7^{\circ}40'57'')$, $(16\text{ h } 45\text{ min } 56.3\text{ s}, -5^{\circ}48'03'')$ and $(16\text{ h } 17\text{ min } 29.6\text{ s}, -41^{\circ}23'44'')$, $(16\text{ h } 16\text{ min } 51.7\text{ s}, -43^{\circ}21'33'')$. The total error box area is 33 arc min². Searches

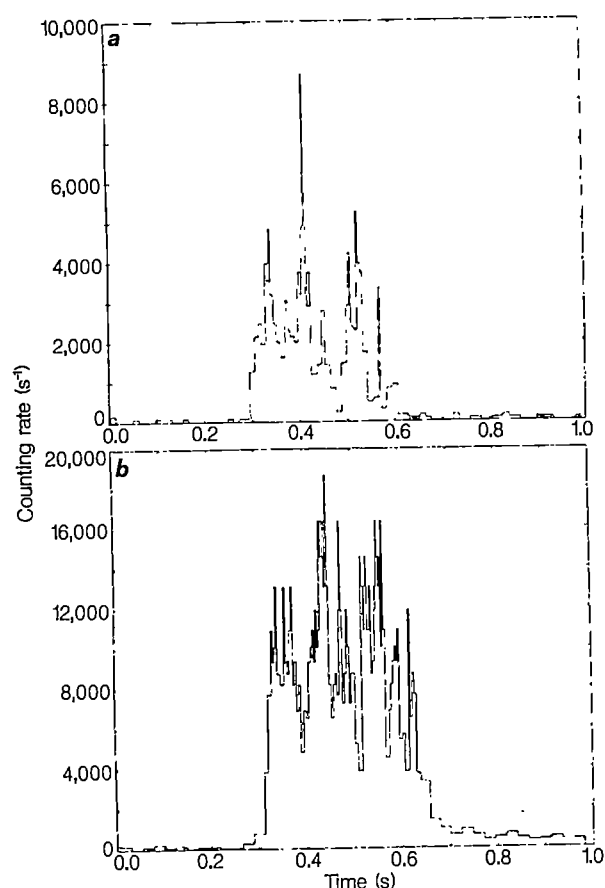


Fig. 1 *a*, ICE time history of GB841215. The energy range is 0.26–2.5 MeV, and the time resolution varies from 0.002 s to 0.012 s. *b*, PVO time history of GB841215. The energy range is 0.1–2.0 MeV, and the time resolution varies from 0.0017 s to 0.012 s.

through various catalogues of γ -ray bursts, pulsars, supernova remnants, flare stars, and high-energy emitters did not result in any source candidates.

We have observed a γ -ray burst with an apparently unique time history. The uniqueness stems from the event's intensity and the rapidity of its temporal structure. However, it is not clear whether the time history is really unique, or whether other events have had similar structure that went undetected due to lack of sufficient event intensity and/or experiment time resolution. If the latter is true, then we must ask if the true 'face' of a γ -ray burst has ever been seen. Are any of our observed γ -ray burst peaks really single peaks? Have any of our measurements (including this one) revealed the underlying fluctuation time scale of a γ -ray burst? Are measured γ -ray burst spectra distorted by pulse pile-up effects? (This could be the case if the photons are emitted preferentially in packets only moderately shorter than the peaks observed in GB841215.) A more detailed discussion of these and other points, as well as a detailed spectral and temporal analysis of this event, will be presented elsewhere.

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Change of solar oscillation eigenfrequencies with the solar cycle

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Solar acoustic eigenfrequencies depend on the internal structure of the Sun, which may change during the 11-yr cycle of magnetic activity as a result of various effects associated with the solar dynamo. Observations of low-degree acoustic frequencies were made, using the ACRIM instrument on the Solar Maximum Mission (SMM) satellite, in 1980 (near solar maximum) and 1984 (near solar minimum). The analysis of these data, presented here, indicates that the frequencies of $l=0$ and $l=1$ acoustic modes in the 5-min band have decreased from 1980 to 1984, by $\sim 0.42 \mu\text{Hz}$ or 1.3 parts in 10^4 . This finding may have important implications for our understanding of the mechanism of the solar activity cycle.

The ACRIM solar total irradiance data have been described in detail elsewhere¹. For the analysis of 5-min oscillations we use a series of flux estimates obtained at the rate of 1/131.072 s, as defined by the periodic chopping of the solar input to the ACRIM sensor by mechanical shuttering. These data are interrupted by the passage of the SMM into the Earth's shadow about every 96 min and by other, apparently random, data gaps.

Data of sufficient quality for studying the 5-min acoustic (p -mode) frequencies were obtained during 1980 from 18 February to 1 December and in 1984 from 1 May (following the successful repair of SMM) to 31 December. Data obtained in 1985 have not yet been analysed.

Frequencies of low-degree p -modes were derived for both the 1980 and 1984 epochs, by means of discrete Fourier transforms of the data from each epoch. The smoothed periodogram of the 1984 data, for example, is shown in Fig. 1. Figure 2 shows the frequency difference (1980 minus 1984) for the nine strongest oscillation peaks in the ACRIM power spectrum. With one exception, the 1980 frequencies of these modes are higher than their 1984 frequencies. To quantify this trend, we assume that all true frequency differences are identical, and that the error distribution is the same for all measured modes. Then we calculate the mean frequency difference, and estimate its error from the standard deviation of the mean of the points:

$$\Delta\nu = 0.42 \pm 0.14 \mu\text{Hz} \quad (1)$$

The 1980 and 1984 observing windows differ in their details. In particular, data from days 243–273 of 1984 were not available

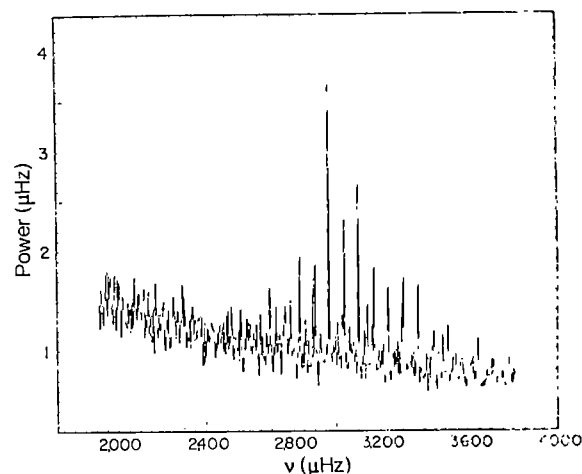


Fig. 1 Smoothed periodogram of 1984 ACRIM data in the 5-min band, showing solar oscillation modes of degree $l=0, 1$ and 2 , and radial order n in the range ~ 17 – 26 . The unit of power is mean square fractional irradiance variation.

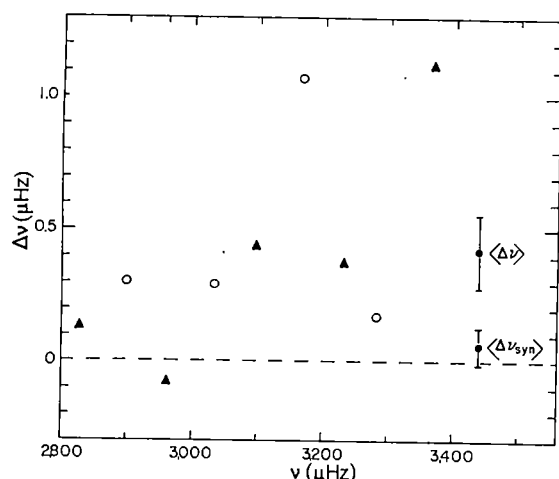


Fig. 2 Measured frequency differences (1980 minus 1984) for individual 5-min solar acoustic modes of degree $l=0$ (○) and $l=1$ (▲), plotted against the frequencies of the modes. The mean and standard deviation of the mean of these nine measurements are indicated by the point labelled $\langle \Delta \nu \rangle$ (equation (1)). The estimated frequency pulling due to the different observing windows (1980 minus 1984) is shown by the point labelled $\langle \Delta \nu_{\text{syn}} \rangle$ (equation (2)).

at the time of the analysis. To test for possible systematic frequency pulling due to the different window functions, we manufactured and analysed time strings sharing properties with the real data. Each synthetic time string is the superposition of variations due to 75 individual oscillation modes plus random variations representing noise. The variation corresponding to an individual mode is patterned after the response of a damped simple harmonic oscillator to a random driving function. The amplitude of the variations representing both those modes seen in the ACRIM data set and random noise was chosen to match the corresponding observed amplitudes in that data set. The amplitude and frequency of the remaining modes, not detected in the ACRIM data, were estimated from velocity oscillation data².

Five independent strings, or runs, of synthetic data were generated. Each run is a separate realization of a statistical process, defined by parameters such as resonant frequencies, linewidths and amplitudes, whose values are the same for all runs. Each randomly generated time series was masked by both the 1980 and the 1984 observing windows and the resulting pair of intermittent data sets from each run was analysed in the same manner as the real data. We therefore obtained five independent estimates of possible frequency shifts due to differences in the window function. From the mean and standard deviation of the mean of five measured shifts, we obtain an estimate of this systematic frequency shift

$$\Delta \nu_{\text{syn}} = 0.06 \pm 0.07 \mu\text{Hz} \quad (2)$$

consistent with no systematic shift. We may obtain a more conservative estimate of the frequency shift of the solar oscillations by subtracting the estimates (1) and (2) and combining their errors quadratically, to give

$$\Delta \nu = 0.36 \pm 0.15 \mu\text{Hz} \quad (3)$$

We have also checked the consistency of the frequencies by comparing portions of the 1984 time series with the whole of the 1980 data. Portion *a* consists of approximately the first 100 days of available 1984 data and portion *b* approximately the final 100 days of that year. The measured frequency shifts (with notation identical to that of equation (1)) are:

$$\Delta \nu_a = 0.43 \pm 0.19 \mu\text{Hz} \quad (4a)$$

$$\Delta \nu_b = 0.36 \pm 0.13 \mu\text{Hz} \quad (4b)$$

Thus the magnitude and sign of the frequency change is consistent between the two portions. We conclude that in 1980, near solar maximum, the low-degree *p*-mode eigenfrequencies were

systematically $\sim 0.4 \mu\text{Hz}$ higher than in 1984 near solar minimum. This result is significant at the 2.5 – 3σ level. We note that a similar change for the most prominent $l=1$ mode of velocity oscillations has been reported³; preliminary analysis indicated a decrease of $0.9 \mu\text{Hz}$ for that mode between 1981 and 1983. In addition, an increase of $1 \mu\text{Hz}$ was suggested for $l=0$ (but not $l=1$) modes from 1980 to 1981.

According to asymptotic theory⁴, the frequency of low-degree acoustic modes of high radial order is proportional, to leading order, to

$$\nu_0 = \left[2 \int_0^R \frac{dr}{c(r)} \right]^{-1}, \quad (5)$$

where $c(r)$ is the adiabatic sound speed and R is the radius of the upper reflection point of the waves and is close to the radius of the photosphere. Thus ν_0 is essentially the reciprocal of the sound travel time across the Sun. The modes used in our analysis (see Fig. 2) have an average frequency of $\sim 3,100 \mu\text{Hz}$. Thus, the frequency change $\Delta \nu$ implies that in 1980, near solar maximum, the sound travel time was shorter by ~ 1.3 parts in 10^4 than in 1984, near solar minimum. A plausible explanation⁵ for this result is that, to an order of magnitude, a similar fractional change in solar radius has occurred, so that the radius is some 10^2 km smaller at solar maximum than at solar minimum. There is other evidence for activity cycle-related changes in the solar radius, from historical meridian circle observations, transits of Mercury and eclipse timings⁶, this evidence also suggests a smaller solar radius at activity maximum, by about the same amount.

It is tempting to associate the observed change in solar *p*-mode eigenfrequencies with a restructuring of the outer convective envelope of the Sun accompanying the activity cycle, for changes in the observed properties of the convective zone have also been reported in association with activity changes. Specifically, both the small-scale granulation⁷ and the larger-scale supergranulation cells⁸ have been found to be significantly smaller at sunspot maximum than at sunspot minimum. Some theoretical studies⁹ have suggested that dynamo generation of magnetic fields deep in the convection zone could lead to a decreased efficiency of convection. This in turn could produce a decrease in the radius of the Sun and a slight increase in *p*-mode eigenfrequencies¹⁰.

Other effects have been suggested which might cause changes in acoustic mode eigenfrequencies correlated with the activity cycle, above and beyond any changes directly coupled to radius changes. Thus Brown¹¹ notes that, in general, turbulent velocities in the convection zone will decrease acoustic mode eigenfrequencies; a decrease in the amplitude of convective turbulence near solar maximum would then lead to an increased frequency at that time, as observed. Bogdan and Zweibel¹² point out that fibril magnetic fields in the upper convection zone, which should be more prevalent near solar activity maximum, would increase the eigenfrequencies, again as is observed. It appears that detailed modelling of a variety of effects may be necessary to explain the observed behaviour; it is hoped that such investigations will produce new insights into the mechanisms of the solar cycle.

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Ionospheric response to changes in the interplanetary magnetic field observed by EISCAT and AMPTE-UKS

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A major question in solar-terrestrial physics concerns the nature of the interaction between the solar wind and the Earth's magnetosphere and ionosphere, and how this depends on the direction of the interplanetary magnetic field (IMF). We report here simultaneous observations of the IMF by the AMPTE-UKS (Active Magnetospheric Particle Tracer Explorers-UK Satellite), and of plasma flow in the high-latitude ionosphere by the EISCAT radar with better time resolution than has been achieved hitherto. We discuss one event in which a sharp southwards turning of the IMF is followed by the onset of rapid ionospheric plasma flow near local noon, demonstrating the close control of ionospheric flows by the IMF. We discuss the causes of the observed 13-min delay, and study the ionospheric heating that accompanies the acceleration.

The AMPTE-UKS¹ has a perigee of 550 km, apogee of 18.7 Earth radii and period of 43.8 h. In October 1984, apogee was near the noon meridian and the satellite was ideally situated to observe the IMF directly upstream of the Earth's magnetosphere and bow shock. The Imperial College magnetometer was operated for periods of up to 10 h during our experiments, and the

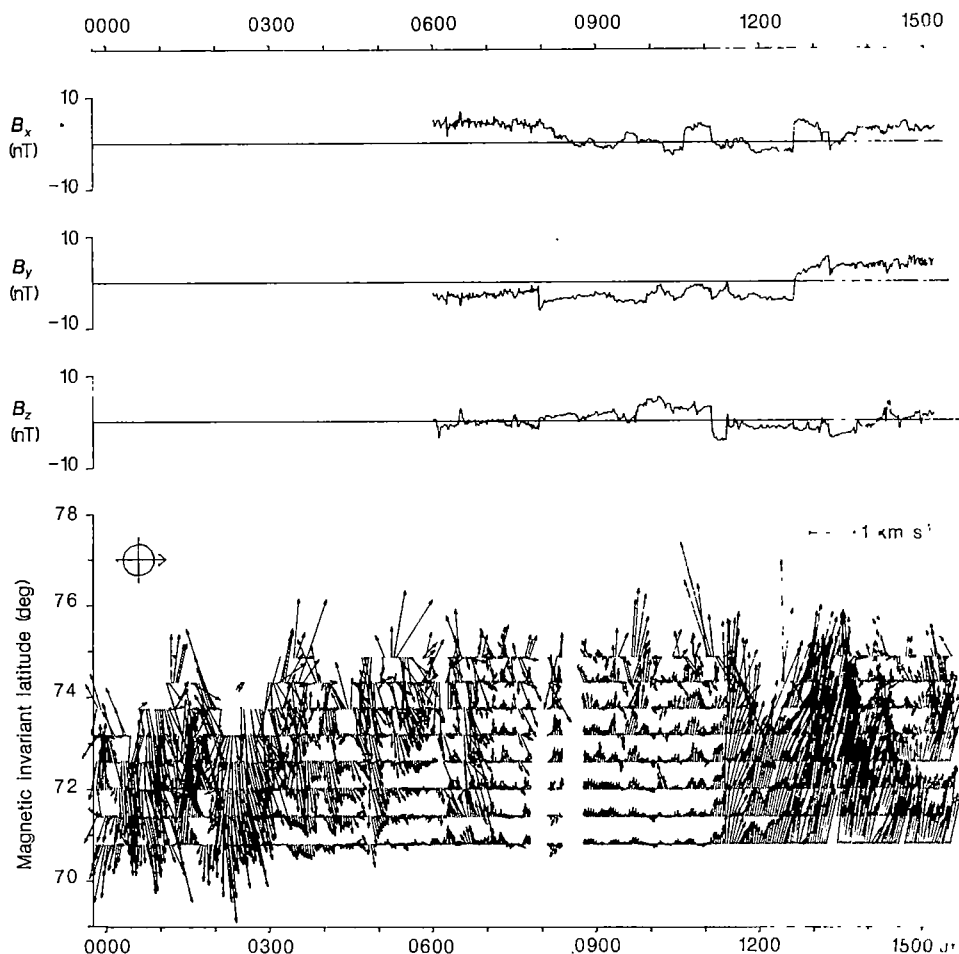
data were displayed in real time at the Rutherford Appleton Laboratory (RAL).

For the EISCAT observations we used the UK-Polar programme² to measure F-region plasma velocity at northwesterly ranges of 525(75)975 km from the UHF transmitter at Tromsø. At such ranges, the lines-of-sight to EISCAT's three receiving stations are so nearly parallel that it is necessary to use 'beam-swinging' to measure vector velocities^{3,4}. To reach high magnetic invariant latitudes Λ (71–75°) we pointed the Tromsø antenna at elevation 21.5°, and recorded data alternately at geographical azimuths 332° and 356° in a 5-min cycle. By combining the line-of-sight components measured in the two beam directions, we obtained two-dimensional velocity vectors, which were plotted at RAL shortly after each measurement. This procedure assumes that the plasma velocity does not vary greatly with longitude over distances of the order of 400 km, nor in about 5 min of time, and that the velocity parallel to the geomagnetic field (**B**) is negligible. At high latitudes the plasma velocity is essentially the $\mathbf{E} \times \mathbf{B}$ drift due to a large-scale electric field **E**, and we believe these assumptions do not seriously influence our conclusions.

The coordinated experiments ran for several hours on 29 September, 25 October and 27 October 1984. Here we discuss features of particular interest seen on 27 October, a day of fairly low geomagnetic activity ($\Sigma K_p = 16+$). Figure 1 shows the data sets covering the periods 0600–1510 UT for AMPTE-UKS and 0000–1530 UT for EISCAT. To minimize congestion of the vectors, we plot the EISCAT data in 'electric field' format, in which upwards on the diagram represents a magnetically northward electric field, corresponding to westward plasma drift velocity.

In high latitudes, the ionospheric plasma convection usually has a twin-cell pattern, consisting of a day-to-night flow across the polar cap, with return flows outside the polar cap on both dawn and dusk sides^{3–7}. Because EISCAT cannot normally

Fig. 1 Interplanetary magnetic field and ionospheric plasma drift velocity on 27 October 1984. Data from the Imperial College magnetometer on the AMPTE-UKS spacecraft are plotted as 1-min averages of the Cartesian components B_x , B_y , B_z in the GSM system (in which x points towards the Sun, and the x - z plane contains the geomagnetic dipole vector). Plasma velocity vectors measured by EISCAT are plotted with westward drift (corresponding to northward electric field) pointing upwards, northward drift to the right. In the EISCAT field of view, magnetic local time is approximately 2 h ahead of UT.



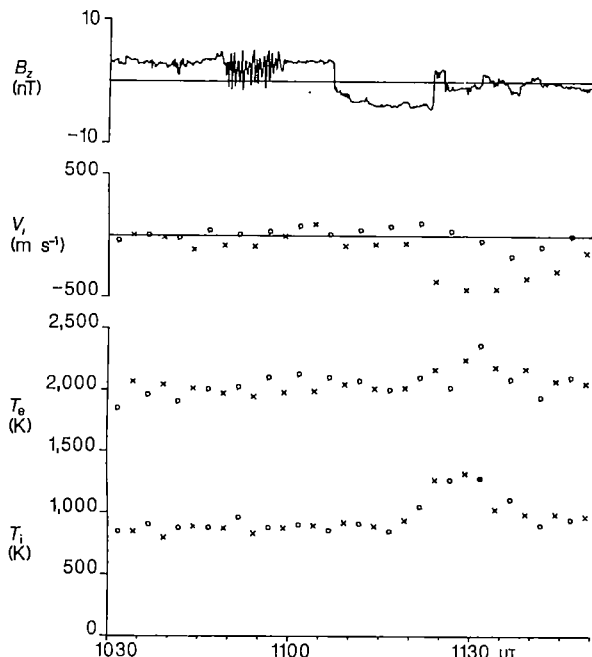


Fig. 2 Data for 1030–1150 UT on 27 October 1984, showing 5-s averages of the B_z component of the interplanetary magnetic field measured by AMPTE–UKS; and ionospheric plasma drift velocity, ion temperature and electron temperature measured by EISCAT at 750 km range, 311 km height, invariant latitude 72.6° (\times , Geographical azimuth 332° ; \circ , azimuth 356°). The 40-s oscillations in B_z between 1049 and 1059 UT are probably 'upstream waves' associated with ions reflected from the bow shock¹⁴. According to a recent recalibration, the values of B_z shown in this figure should be reduced by 1 nT.

observe the polar cap by day, our measurements mostly show the return flows, eastward in the morning and westward in the evening. Depending on solar–geophysical conditions, the reversals between eastward and westward flow may occur some hours away from noon and midnight.

From 2350 UT we see predominantly fast eastward plasma flow ($1\text{--}2\text{ km s}^{-1}$), associated with the dawn side of the convection pattern. Between 0230 and 0310 UT this fast-flowing region recedes across the field of view, the poleward speed of the boundary being $\sim 150\text{ m s}^{-1}$ which could be produced by the Earth's rotation beneath a fixed flow pattern. There follow several hours of quieter flow ($0.3\text{--}1\text{ km s}^{-1}$), during which the morning east-to-west reversal of plasma velocity occurs at around 0600 UT. From 0600 the IMF northward component (B_z) fluctuates about zero, becoming positive after 0800 UT.

At 1107 UT the IMF suddenly turns southwards and B_z is mainly negative (0 to -5 nT) for the next 3 h. This change is followed at 1120 UT by a sudden acceleration of the ionospheric plasma flow which we judge to be simultaneous (to within $\pm 1\text{ min}$) over the whole radar field of view. The coherence of the data suggests that the effect is temporal, the plasma being accelerated in $< 5\text{ min}$ to speeds of $\sim 1\text{ km s}^{-1}$ over a range of at least 3° in Λ . In principle, the rapid flow could have started earlier in the auroral oval (probably at $\Lambda \sim 76^\circ$ in quiet conditions⁸) and expanded across the field of view, but that seems unlikely. It would require an equatorward boundary motion of at least 3 km s^{-1} , 10 times what is typically observed⁹. Similar plasma accelerations have been observed by the Sondrestrom radar¹⁰, but with a coarser time resolution of $\sim 25\text{ min}$.

It is reasonable to suppose the southward IMF turning and the acceleration of ionospheric plasma are related, through processes that involve field-line reconnection at the dayside magnetopause. If so, roughly half the observed 13-min delay can be attributed to propagation from the satellite to the ground,

as follows: (1) Travel-time of IMF disturbance from AMPTE–UKS to the subsolar magnetopause: $4 \pm 1\text{ min}$; (2) propagation time of Alfvén waves from the subsolar magnetopause to the cusp ionosphere: $2 \pm 1\text{ min}$.

The other half could be due to three factors. First, the onset of reconnection might be delayed after the arrival of southward-pointing field at the magnetopause, although we do not know why that should happen. Second, several Alfvén transits may well be required to communicate to the ionosphere the full stress imposed by reconnection¹¹. Third, up to 3 min extra delay would occur if (as mentioned above, but considered unlikely) the rapid flow is initiated by an expansion of the auroral oval, instead of being transmitted through the ionosphere in $\sim 1\text{ s}$ at the Alfvén speed. We note that a similar unexplained delay of about 10 min has been found between changes of the IMF B_z and changes of the DP2 current system, as measured by ground-based magnetometers¹². DP2 is the magnetic disturbance associated with the twin-cell plasma convection pattern, which we measure in a more direct way using the radar.

The increase of plasma drift velocity is accompanied by a rise of 400 K in ion temperature between 1120 and 1130 UT (Fig. 2), which we attribute to ion–neutral frictional heating. On the timescale we are considering, the difference between ion and neutral gas temperatures, T_i and T_n , is given approximately by

$$(V_i - U)^2 = (3R/M)(T_i - T_n) \approx 1,300(T_i - T_n) \quad (1)$$

(see ref. 13), where V_i is the plasma drift velocity, U the neutral-air velocity, R the gas constant and M the mean molecular mass of the air (taken as 19 AMU). Assuming that initially $U \approx V_i \approx 200\text{ m s}^{-1}$ and that $T_i \approx T_n$, as seems reasonable, the observed temperature rise of 400 K requires $|V_i - U| \sim 700\text{ m s}^{-1}$, well within our observations. The ion temperature subsequently decreases, presumably because $(V_i - U)$ decays as the neutral air is accelerated by collisions with the drifting ions.

To summarize, we have observed a southward turning of the IMF, followed 13 min later by acceleration of ionospheric plasma. We believe this event demonstrates the connection between ionospheric plasma flows and the IMF direction, although the observed delay is some minutes more than expected on the basis of simple propagation from the satellite to the ground. The ionospheric temperature data support our interpretation. This event is the best isolated southward turning that we observed. We shall discuss elsewhere other, less clear-cut changes of IMF in our data, and we think that the ionospheric response may depend on factors such as local time. We stress the importance of making simultaneous IMF and ionospheric observations with good spatial and temporal resolution.

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Geomagnetic secular variation in Sicily and revised ages of historic lavas from Mount Etna

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The variation of geomagnetic field direction in Sicily during the past 700 yr has tentatively been determined using lavas of known date from Mount Etna¹. Additional palaeomagnetic studies on several hundred volcanic samples, combined with archaeomagnetic investigations carried out on Norman buildings, have improved the previous results and permit a reconstruction of the geomagnetic variation curve to about AD 1000. This curve agrees well with those obtained for other European countries²⁻⁶ and may be used as a reference for checking the ages attributed to archaeological structures as well as volcanic products in southern Italy during the past 1,000 yr. The present results cast serious doubts on the true ages of numerous historically dated lavas from Mount Etna, most of which are at least several centuries older than previously believed. The conclusions have implications for the succession of eruptions, effusion rates, magmatic evolution, and so on, and demonstrate the inconsistency of eruptive models based on historical records alone.

The Etnean lavas from major eruptions during the past 400 yr are well known, the most famous being the 1669 flow that buried about 15 villages and part of the town of Catania. Contrary to popular belief, however, older eruptions are far from being accurately described^{7,8}. Between AD 1600 and 1300, the products of only three eruptive periods can be identified with a reasonable degree of confidence: (1) the 1536 and 1537 flows on the south flank⁹⁻¹¹; (2) the 1408 flow north of Pedara village^{11,12}; and (3) the 1329 cone of Monte Rosso near Fleri (east flank), the name of which is cited in two early manuscripts found by Recupero¹². The palaeomagnetic direction given by welded scoriae from Monte Rosso is consistent with that of a lava flow erupted in 1301-02 on the island of Ischia (Gulf of Naples)¹³: because this eruption is the only one that occurred at Ischia in historical times, its products cannot be confused with others. For com-

parison with Etnean lavas, the magnetic inclination of the Ischia flow has been corrected to the latitude of Sicily.

The lavas mentioned above have been used to reconstruct the geomagnetic variation curve of field direction (Fig. 1), no reference being made to direct measurements as they only extend back to AD 1890 in Sicily. Other flows of supposedly known age, which are shown with quotation marks in Fig. 1, have also been extensively sampled: their peculiar behaviour will be discussed later.

Detailed results and discussion of the techniques of study (such as the removal of viscous remanent magnetization and alternating field demagnetizations) are reported in refs 1 and 7. Note that Etréan lavas are quite sensitive to parasitic magnetizations acquired during sawing or coring. Therefore, we used only 'big' samples (0.5–2 kg) oriented by the plaster method^{1,2}, with azimuth reference from the Sun shadow. This technique also has the advantage of permitting a much better precision during sampling and measurements. Table 1 summarizes the results obtained after magnetic cleaning from lavas of both reliable and dubious dates. The semi-angle of the 95% confidence cone of the average flow directions ranges from 0.71 to 1.76°, thus giving a precision similar to that of usual archaeomagnetic data.

Figure 1 shows that the points corresponding to all the recent flows (AD 1910 to 1610) reproduce the curve of geomagnetic secular variation known from direct measurements in Paris and London^{2,3}, allowing for difference in latitude. By going further back, there is an increasing number of flows (indicated between quotation marks) whose palaeomagnetic directions are inconsistent with that of the geomagnetic field expected at the respective dates. The eruptions to which these lavas were ascribed are poorly located by the historical data. Sometimes, re-examination of early documents enables us to preclude the dates attributed—later—in the modern literature. For example, the “1595” flow, which corresponds to two petrologically distinct flows⁷—“Gallo Bianco East” and “Gallo Bianco West”—in Fig. 1, is undated in documents published before the middle of the nineteenth century. Furthermore, contemporaneous witnesses pointed out that Etna had been inactive for at least 30 yr before 1603 (refs 11, 14). The “1595” date is only quoted by Sartorius in his *Atlas of Etna*¹⁵, although this author does not mention any eruption for this particular year¹⁶. It would appear that “1595” is a printing error. A full discussion of the problems related to spurious dates attributed to Etnean lavas is given elsewhere^{7,8}.

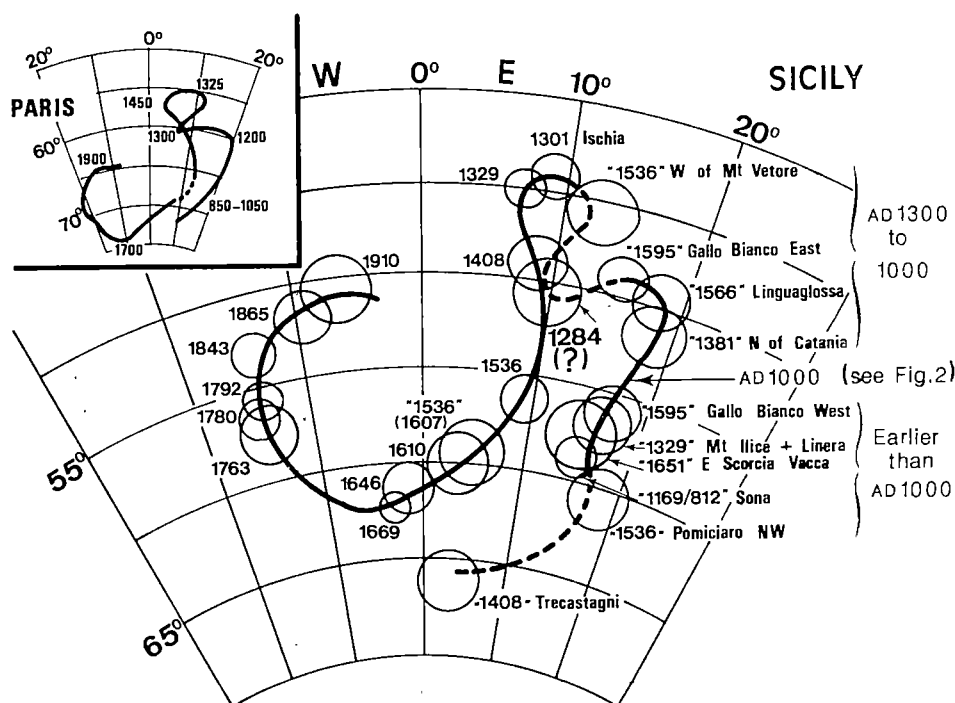


Fig. 1 Geomagnetic variation in Sicily recorded in historically dated Etnean lavas. Ovals indicate the 95% confidence intervals. The dashed lines express uncertainties on the trend of the curve, 1284(?) and "1408" (Trecastagni) being possibly of prehistoric age. Dates in quotation marks are erroneous, although the morphology of the flows indicates a very recent age (see text). Note the general agreement with the archaeomagnetic curve obtained in France (upper left, from refs 2, 17).

Table 1 Palaeomagnetic directions of lava flows or cones of known (a) or presumed (b) ages

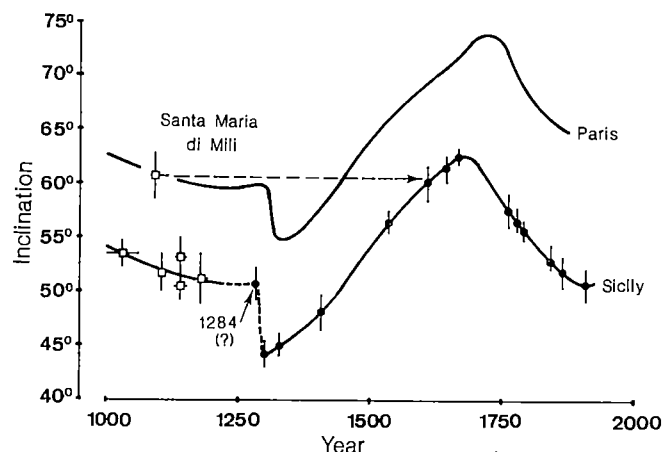
a, Volcanic units of known age	N	\bar{I} (deg)	\bar{D} (deg)	α_{95}	k
1669 I	7	61.7	-3.0	0.97	2,963
1669 II+III	7	62.3	-3.6	2.29	535
1669 IV	5	62.7	-3.0	1.62	1,503
1669 V	6	62.6	-2.7	1.35	1,793
1669 VI	5	63.8	-3.3	1.28	2,395
1669 (mean)	30	62.5	-3.3	0.71	1,310
1910	11	50.7	-6.9	1.53	760
1865	18	51.9	-9.5	1.41	583
1843	12	53.2	-14.2	1.12	1,552
1792	23	55.8	-14.2	1.01	837
1780	32	56.7	-14.9	1.04	582
1763	6	57.6	-14.4	1.51	1,437
1646	8	61.3	-1.5	1.25	1,564
1610	10	60.0	3.5	1.61	750
1536, Piano San Leo	21	56.5	8.7	1.04	855
1408, Pedara	12	48.1	7.9	1.67	703
1329, Mt Rosso, Fleri	10	45.1	7.1	1.04	1,824
1301-1302, Ischia	24	44.2	9.0	1.20	566
1284(?) Petrulli-Monacella	18	50.7	9.7	1.52	468
b, Volcanic units of presumed age					
"1651", Scoriavacca	12	57.5	14.7	1.75	581
"1595 I", Gallo Bianco West	16	56.0	17.0	1.44	591
"1595 II", Gallo Bianco East	10	48.9	15.1	1.20	1,351
"1566", Linguaglossa	19	49.7	17.7	1.40	525
"1536 I", West of Mt Vetore	4	45.7	12.6	1.76	1,583
"1536 II", Lower Pomiciaro (north-west)	9	60.7	18.3	1.58	874
"1536 III", Upper Pomiciaro	7	59.4	4.8	1.67	1,172
"1408", Trecastagni North	10	66.1	3.0	1.52	1,060
"1381", Gravina di Catania	12	51.1	18.6	1.41	815
"1329", Mt Illice, Linera	15	56.7	16.4	1.44	731
"1169", Aci Castello	11	57.3	8.2	1.24	1,165
"1169 or 812", Sona (south-west)	8	58.7	14.8	1.04	2,578

N is the number of samples for each eruptive unit (for the 1669 flows, the detail of each site of sampling is shown); α_{95} and k are the Fisher 95% confidence limits and precision parameters, respectively. \bar{I} is the mean inclination and \bar{D} the mean declination.

Thus, most of the lavas believed to have erupted between AD 1651 and 1284 are actually older than this period. The 1284(?) flow itself is problematic; both petrographic and palaeomagnetic evidence⁷ show that it consists of several flows of undoubtedly different ages in the area indicated as a single "1284" lava by Sartorius¹⁵. One of these flows has a palaeodirection that is not inconsistent with the trend of the geomagnetic variation expected for this late thirteenth-century period (compare with Paris and Oxford, Fig. 1 and refs 3, 17). However, its chemical composition and mineralogy are close to those of some prehistoric lavas, and its morphology is less preserved than for any of the historical flows. Thus, there is no objective argument to ascertain whether it could represent the 'true' 1284 lava.

The other flows of presumed age, in contrast, have a well-preserved morphology and petrological characteristics similar to those of well-dated lavas. They probably belong to unrecorded eruptions that occurred during the medieval period, an assumption which is consistent with the results of archaeomagnetic records obtained in other parts of Europe^{2-6,17}. These Etna flows can be used, therefore, to reconstruct the trend of the geomagnetic curve (right-hand side of Fig. 1), although its variation as a function of time cannot be determined, owing to the lack of historical records.

For dating these Middle Age flows of Etna, at least approximately, a preliminary archaeomagnetic study has been carried out on bricks from the walls of six Norman buildings in Sicily (Table 2). Alternating-field cleaning and thermal demagnetization techniques² were applied to the brick samples; the result was an improvement of the 95% confidence intervals, while the mean inclination remained virtually unchanged. The curve of

**Fig. 2** Variation of geomagnetic inclination in Sicily during the past 1,000 yr, from lavas (solid symbols) and bricks (open symbols). The variation of inclination at Paris^{2,17} is shown for comparison.**Table 2** Geomagnetic inclination recorded in bricks from Norman buildings in Sicily

Site	Date (AD)	No. of samples	\bar{I} (deg)	Treatment	Observations
Santi Pietro e Paolo di Agro	≈1000-1060	43	53.5	—	Bricks (church constructed before the Norman period)
		41	53.0 ± 1.4	15 mT	
Santa Maria di Mili	"1092"	25	60.7 ± 2.1	—	Bricks (foundation of church, 1092; possible restoration ~1542)
Santissimo Salvatore di San Marco d'Alunzio	1105	31	51.8 ± 1.7	—	Bricks (monk Gregorio's testament: foundation of church 1105)
Palazzo Reale (Palermo)	1130-1150	47	50.5 ± 1.3	—	Bricks (Pisan Tower windows)
Cefalù	1132-1148	17	53.5 ± 2.1	—	Bricks from the south nave of the church
		17	53.2 ± 1.8	150 °C	
Monreale (Palermo)	1172-1185	17	51.8 ± 2.6	—	Bricks (ornamental material from terrace)
		17	51.2 ± 2.3	140 °C	

geomagnetic inclination obtained (Fig. 2) is very similar to that observed in France (refs 2, 17 and I.B., unpublished data), with the difference of latitude between the two countries taken into account. An unexpected archaeological result concerns the Santa Maria di Mili church: although built in AD 1092, the church has clearly been restored during the sixteenth century, as revealed by the strong magnetic inclination carried by all the bricks sampled.

This new inclination curve for Sicily shows that the supposed Etna flows of "1595" (Gallo Bianco East), "1566" (Linguaglossa) and "1381" (north of Catania) have palaeomagnetic inclinations consistent with eruptions occurring at ~AD 1050-1250. Other flows (lavas from Gallo Bianco West, Mount Illice, Scoriavacca, Sona, Pomiciaro north-west and Trecastagni north) are significantly older, probably between AD 700 and 1000, judging

from the strong increase of inclination recorded at this epoch in Europe^{2-6,17}.

We hope to improve our knowledge of the geomagnetic variation in Sicily by investigating the archaeological structures dated from the Greek civilization to the Roman and Byzantine periods. The resultant curve could be used to check the true age of numerous eruptive products from South Italian volcanoes, as well as archaeological structures of uncertain date.

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A dynamic model of the curvature of the Mariana Trench

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Oceanic trenches are prominent features of the Earth's surface. They represent the convergent plate boundaries and generally possess convex curvatures towards the subducting plates. Based on this observation, many static geometric models have been proposed for the origin of trench curvatures¹⁻⁶. Unfortunately, many of these models have limited applicability⁷. Alternatively, a dynamic model has been proposed which suggests that trench curvatures are evolved from collisions between aseismic ridges and the trench axis⁸. We have developed a flow model to quantitatively evaluate this dynamic process for the Mariana Trench system. The model is able to yield an excellent fit to the present trench curvature and determine past rotations for back-arc volcanic islands such as Saipan and Guam with surprising accuracy when compared with palaeomagnetic observations.

The Mariana Trench represents the convergent boundary between the East Philippine plate and the subducting Pacific plate. It is bounded to the north by the Marcus-Necker Ridge and to the south by the Caroline Ridge (Fig. 1). Both ridges are classified as aseismic ridges because of their elevated bathymetry and their lack of large seismic activities^{9,10}. These ridges are more buoyant relative to their adjacent oceanic sea floor due to either thermal (for example, higher temperature) or chemical (for example, thicker crust) effects. Because of their stronger buoyant character, they tend to resist subduction⁸. As a result, these aseismic ridges will continue their westward migration while other parts of the ocean floor begin their descent into the mantle. Thus, an initially straight trench will evolve into a convex geometry towards the subducting plate. In this scenario, the overriding plate is assumed to be stationary while the subducting plate is converging. However, the same problem can be formulated in which the subducting plate is chosen to be stationary

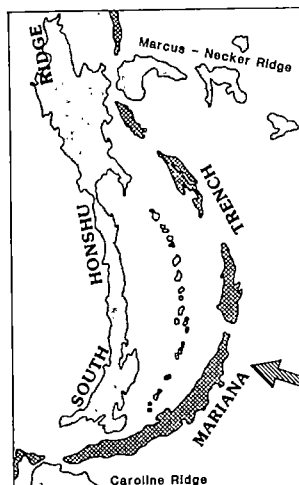


Fig. 1 Tectonic setting of the Mariana Trench.

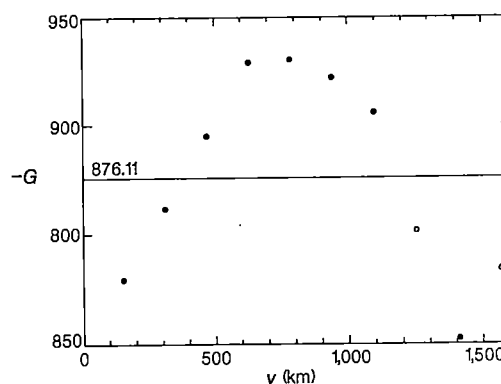


Fig. 2 Observed values of G as a function of y where y is defined in Fig. 3. The solid line represents the mean value for G .

while the overriding plate is flowing over it. As far as the plate boundary geometry is concerned, these two reference coordinates are interchangeable. However, the latter view provides some mathematical simplicities in formulating the problem.

Consider a newtonian viscous fluid with a linear front moving over two stationary point obstacles. The obstacles represent the leading edges of the aseismic ridges as shown in Fig. 1. Since viscosity of geological materials is quite high especially in surface conditions, surface deformation will obey Stokes' flow where inertia forces can be neglected. The governing equation is then given by

$$0 = -\frac{1}{\rho} \frac{dP}{dx} + \nu \frac{d^2 u}{dy^2} \quad (1)$$

where dP/dx is the horizontal pressure gradient arising from stress imbalance within the overriding plate, possibly due to the unboundedness of the trench wall, and it is assumed to be constant; ν is the kinematic viscosity and u is the flow velocity. As the obstacles are stationary, boundary conditions require vanishing velocity at $y=0$ and $y=L$. L is the separation between the obstacles. Consequently, equation (1) can be integrated twice to obtain a velocity profile which in turn can be integrated again with respect to time to yield a curvature profile as given below, where μ is the viscosity of the fluid layer:

$$x = \frac{1}{2\mu} \frac{dP}{dx} (y^2 - Ly)t \quad (2)$$

This equation describes the evolution of a trench curvature according to the model of Vogt *et al.*⁸.

As there are some parameters in the model which are not very well constrained, we shall first attempt to use observed data to determine the acceptable range of values for these parameters. Later, we shall demonstrate that values thus determined are able to fit another set of geophysical data. By doing so, the validity of our model can be established.

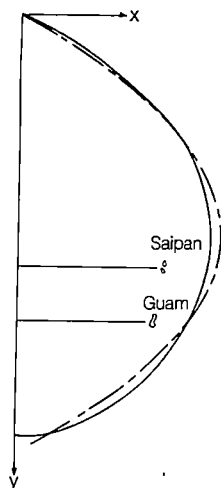


Fig. 3 Curvature of the Mariana Trench. The solid line is the observed curvature while the dashed line is the theoretical curvature based on the mean value of G as determined in Fig. 2.

Evolution of the Mariana Trench has been studied by many investigators^{11,13}. The collision between the aseismic ridges and the proto-Mariana trench is set at approximately the middle Miocene time. While some constraints can be established for the timescale, very little can be said about the newtonian viscosity of the surface layer or the pressure gradient across a trench, which are the other two parameters intrinsic to the model. Fortunately, these three parameters can be combined to form a single parameter group [that is, let $1/G = (1/2\mu) (dP/dx)t$]. We shall test if the observed Mariana Trench curvature is able to yield a constant G . Taking the separation between the two aseismic ridges to be $\sim 1,650$ km, the observed values for G are given in Fig. 2. The mean value for this data set is -876.11 km. Based on this mean value, a theoretically predicted trench curvature can be constructed according to equation (2). Comparison of the predicted and observed profiles is given in Fig. 3. Agreement between the model and observation is quite good.

To demonstrate the validity of this model, we shall next examine the relationship between this model and some palaeomagnetic studies behind the Mariana Trench. Application of palaeomagnetic data to study plate rotation has been carried out by many investigators^{11,14-17}. Palaeomagnetic declinations of volcanic rocks of different ages can be measured at the same location. The discordance of palaeomagnetic direction will yield information on the sense and magnitude of rotation for the sampled localities. McCabe¹¹ has compiled some palaeomagnetic data for the southern Mariana Islands. Our model predictions will be compared with these data.

Investigation of a fluid particle rotation within a flow such as the one described previously, requires the theory of finite deformation¹⁸. Geological applications of finite deformation theory have been carried out by Elliot¹⁹ and McKenzie²⁰. In our case, as the flow field is very similar to a steady simple parallel flow, the rate of finite rotation of any fluid particle within the flow can be described by a linear combination of shear strain and fluid vorticity¹⁹, where e is shear strain and ω is vorticity.

$$\dot{\theta} = e + \omega = \frac{du}{dy} = \frac{1}{2\mu} \frac{dP}{dx} (2y - L) \quad (3)$$

Consequently, the finite angle of rotation can be written simply as the time integration of equation (3).

$$\theta = \frac{1}{2\mu} \frac{dP}{dx} (2y - L)t + \theta_0 \quad (4)$$

where θ_0 is the initial angle with respect to the magnetic north prevalent at the time of the collision. Equation (4) can also be derived using a more rigorous approach as described in refs 18, 20. Based on equation (4), finite rotation of the volcanic islands

can be calculated. Note that the time variable t here is not the same as that in equation (2) for trench curvature determination; it is the time that is required for the island to travel from the point when it crossed the line connecting the two obstacles to its present position. Given the flow velocity and the distance, the time duration can be estimated. As a result, rotation of volcanic islands as a function of time can be obtained, and is plotted in Fig. 4; also given are the palaeomagnetic rotation data for Guam and Saipan Islands as compiled by McCabe¹¹. Agreement is surprisingly good.

As indicated in Fig. 3, a parabolic profile is adequate to fit the Mariana Trench. Based on a mean value of -876.11 km for the grouped parameter G , a fit between the theory and the observation can be obtained with a standard deviation of $<5\%$. A closer examination of the comparison indicates that the mismatch is not randomly distributed. The theory over-predicts movement near the centre part of the trench while underestimations occur near the aseismic ridges. One possible reason for this discrepancy may be the existence of a small component of non-newtonian rheology within the overriding plate, since power creep laws will tend to flatten a parabolic profile as evidenced in glacial flow²¹ and in other non-newtonian channel flows²². The non-newtonian contribution appears to be small, however, since the match by a newtonian theory is quite sufficient. Alternatively, the bathymetric trench axis may not represent the actual convergent boundary due to the accumulation of sediments in the accretionary prism²³. Unfortunately, the actual boundary geometry can only be mapped with dense coverage of seismic reflection experiments.

Based on tectonic reconstruction studies, the initial collision between the aseismic ridges and the Mariana Trench has been estimated to have occurred at about the middle Miocene^{11,13}. We thus assign a 15 Myr numerical value for computational purposes. It follows, therefore, that the mean value for $(1/2\mu)/(dP/dx) = 2.4 \times 10^{-21} (\text{m s})^{-1}$. Using this best-fit value, we are able to calculate the velocity of the two volcanic islands (Guam and Saipan), where palaeomagnetic rotation data are available for our model comparison. Table 1 gives the values for velocity and elapsed time for the two volcanic islands. Using

Table 1 Parameter values used for palaeomagnetic rotation calculation

	Guam	Saipan
θ_0 (deg)	-19°	-19°
L (km)	1,650	1,650
Time since collision (Myr)	15	15
x (km)	515.63	556.88
y (km)	1,155.00	948.75
Velocity (km Myr ⁻¹)	43.31	50.88
Time since rotation started (Myr)	11.91	10.95
θ (deg)	-53.26°	-31°

these values, the rotational history for Guam and Saipan can be calculated. Excellent agreement between our model predictions and the observations is clearly demonstrated in Fig. 4. In addition, our model is able to confirm the fact that rotation at Guam is more intense than that at Saipan. It is simply because Guam is closer to the Caroline Ridge. As a result, more vorticity and shearing are expected. Saipan, on the other hand, is closer to the midpoint between the two aseismic ridges where flow is more uniform and with vanishing shear and vorticity. Note that the amount of rotation as determined by our model depends only on the time interval between the present and the time at which the volcanic islands flowed past the leading edges of the aseismic ridges, if the rocks were formed earlier. If rock samples were formed after that time, rotation is calculated based on the time interval between the present and the time at which the rocks were solidified. Consequently, rotation of younger rocks can also be calculated. Unfortunately, data for younger volcanic rocks are not sufficient for a meaningful comparison. Of course, maximum rotation behind the migrating trench will occur in the mid-Miocene volcanics. Rocks with ages older than mid-

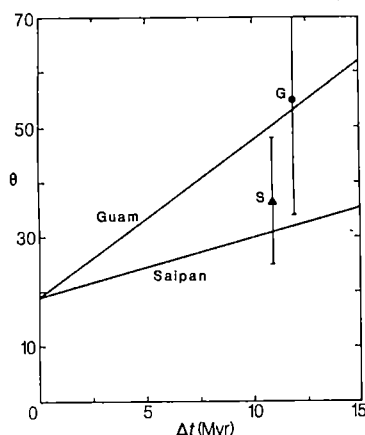


Fig. 4 Palaeomagnetic rotation as a function of time for Guam and Saipan islands. Observed rotations (●, ▲) as reported in ref. 11 are also plotted.

Miocene are not expected to show any larger rotation, since the trench is hypothesized to have moved uniformly before the collision with the two aseismic ridges. Because of the excellent agreement between our model and the palaeomagnetic data, the proposed dynamic model for the curvature of the Mariana Trench is believed to be valid. The model is especially attractive because of its ability to explain the differential rotation between Saipan and Guam.

The remaining issue is whether the value of 2.40×10^{-21} chosen for $(1/2\mu)/dP/dx$ is reasonable. To address this question, it is necessary to know the viscosity of the surface layer and the pressure gradient within the overriding plate; neither parameter is very well understood, thus one can only examine the range of possibilities. If the pressure gradient is of the order of a few hundred bars over 1 km, then the viscosity will be about 10^{26} . A pressure difference of a few hundred bars is believed to be reasonable as this is about the strength of surface rocks. Is the required viscosity too low? Although very few studies have been carried out to estimate the viscosity of the surface layer, some laboratory observations have been made in an attempt to provide answers to this question²⁴. Based on Ito's experiment on creep deformation of a granite slab over a 10-yr period²⁴, he found that the deformation of his granite slab can practically be described by a newtonian rheology with a viscosity of $\sim 10^{23}$ Pa s. Thus, our implied value for surface rock rheology is not unreasonable in view of the values derived from Ito's experiments.

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Palaeobiological and sedimentological implications of fossil concentrations

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Concentrations of fossil hardparts are common features of the stratigraphical record and are preferred collecting sites for most palaeontological data. Nonetheless, most investigations into the nature of the fossil record have analysed the biasing effects of selective hardpart transport and destruction^{1,2} rather than the consequences of the concentration process itself. Genetic classification is discouraged by the diverse origins of skeletal accumulations, which range from predator gastric residues to shelly shoals and biostratigraphically condensed deposits; concentrations can thus form over time intervals of a few minutes to hundreds of thousands of years. I show here that the close association of shell beds with stratigraphical discontinuities in Miocene shallow marine deposits of Maryland^{3,4} provides the basis of a model of skeletal accumulation cast entirely in terms of changes in net sedimentation. This simple sedimentological model is a surprisingly powerful predictor of post-mortem bias and ecological composition of fossil assemblages, suggesting that fossil-rich and fossil-poor strata are qualitatively different, both as repositories of palaeontological information and as settings for biotic interactions. Moreover, the apparent primary importance of rates of sedimentation in skeletal accumulation—despite emphasis usually placed on rates of hardpart input—suggests a new approach to inferring the detailed dynamics of sediment deposition and erosion in the formation of stratigraphical sequences.

Description of the upper and lower contacts of shell (or bone) beds as sharp or gradational has genetic significance because bed contacts not only describe the physical relation of the shell bed to adjacent beds, but also relate the process of hardpart concentration to processes responsible for the accumulation of the surrounding, less fossiliferous sediment. Except for contacts created by bioturbation or diagenesis alone, sharp contacts indicate disjunct shifts in sedimentation and usually record an

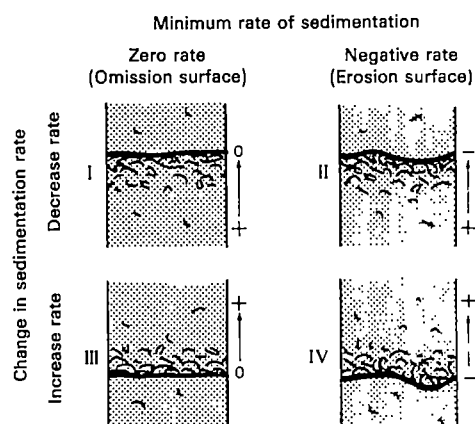


Fig. 1 Field classification of shell beds on the basis of stratigraphical contacts. Type I shell beds grade from less fossiliferous sediment and terminate in a sharp omission surface; type II shell beds also grade upwards with increasing shell-packing density but terminate along an erosion surface; type III shell beds rest on an omission surface and grade upwards into less fossiliferous sediment; and type IV concentrations have erosional basal contacts and grade upwards. Concentrations associated with such surfaces must arise in the context of some change in net sedimentation, since discontinuities indicate conditions of zero (omission) or negative (erosion) net sedimentation, and sediment between discontinuities records positive net sedimentation (deposition).

Table 1 Taphonomic and palaeobiological predictions for four simple patterns of fossil accumulation as modelled in Fig. 1

Shell bed type	Modelled change in sedimentation	Hardpart residence time on seafloor	Hardpart abundance on seafloor	Severity of post-mortem bias					Community composition			Species morphology		
				Physical abrasion & fragmentation	Bio-erosion	Dissolution	Transport addition or removal	Time-averaging	Deep or mobile infauna	Encrusting & boring taxa	Shell gravel taxa	Shell gravel morphs	Variance owing to: Time-averaging	Selective destruction
I	Decrease from + to 0	Increase	Increase	Increase	Increase	Increase	+/- Increase	Increase	Decrease	Increase	Increase	Increase	Increase	+/- Decrease
II	Decrease from + to -	Increase	Increase	Strong increase	Increase	Increase	Increase	Strong increase	Decrease	Increase	Increase	Increase	Strong increase	Decrease
III	Increase from 0 to +	Decrease	Decrease	Decrease	Decrease	Decrease	+/- Decrease	Decrease	Increase	Decrease	Decrease	Decrease	Decrease	+/- Increase
IV	Increase from - to +	Decrease	Decrease	Strong decrease	Decrease	Decrease	Decrease	Strong decrease	Increase	Decrease	Decrease	Decrease	Strong decrease	Increase

Hardpart abundance and hardpart residence time at or near the sea floor are treated as simple functions of rate of sedimentation (hardpart input is assumed to be constant); fossil assemblages sampled from different horizons within any particular shell bed will be characterized by different degrees of post-mortem bias and patterns of biotic response.

episode of erosion or omission (non-deposition)⁵⁻⁷. Hardpart concentrations associated with sharp discontinuities thus must have formed in the context of some change in net sedimentation. These concentrations can be divided into four basic types (Fig. 1), depending on whether hardparts lie on top of or directly underneath the discontinuity surface (as in the "Sohlbanke" and "Dachbanke" of Brinkmann⁸), and whether the surface exhibits scour and truncation features of erosion (negative sedimentation rate) or merely omission (zero net sedimentation rate). As an initial simplifying assumption, hardpart input is assumed to be constant throughout the accumulation period; the model is later shown to hold even after relaxation of this unrealistic assumption.

Type I and type II shell beds (Fig. 1) indicate hardpart accumulation during a slowing down in net sedimentation from high initial rates which exceed hardpart input. Hardpart packing density increases upwards as sedimentation approaches a zero or negative net rate. Type III and type IV beds record an acceleration in sediment accumulation from initially very low rates that foster the concentration of hardparts towards higher positive rates which exceed the background rate of hardpart input. These dynamics of sedimentation have immediate implications for assemblages collected from both fossil-rich and fossil-poor strata.

As rates of sedimentation decrease during the formation of types I and II shell beds, individual hardparts are exposed at the depositional interface for increasingly long periods of time. Assemblages collected from successively higher horizons within the shell bed should thus exhibit a higher frequency and severity of shell damage by abrasion, fragmentation and bio-erosion (Table 1). This trend would be reversed in types III and IV beds. In addition to suffering more pronounced bias owing to selective destruction of hardparts, assemblages that accumulate during the phase of lowest net sedimentation are also more likely to be time-averaged, that is, composite records of a series of populations that occupied the site at different points in time. Time-averaging should be more severe in types II and IV beds than in types I and III beds because of vertical mixing of faunas during erosional reworking. Types II and IV beds are also most likely to be biased by the addition and removal of hardparts through selective transport.

By controlling rates of hardpart burial, net sedimentation also determines the abundance of hardparts in the substratum and thereby influences the physical characteristics of benthic habitats. Ecologically, type I shell beds record the transformation of an initial soft-bottom habitat into an increasingly shelly and thus coarser-textured, firmer and topographically more complex substratum. This change in the physical environment facilitates colonization by borers and encrusters of dead hardparts and by free-living and attached epifauna which prefer or require stable substrata⁹. At the same time, the *in situ* development of a shell gravel inhibits mobile and sedentary burrowers which occupied

the original soft sediment habitat⁹. Types I and II beds should thus exhibit an upward increase in the abundance and diversity of epifaunal species and a concomitant decrease in the numbers of mobile and of especially large or deep-burrowing infauna (Table 1). Assemblages from the most densely fossiliferous parts of the beds will be characterized by ecologically mixed assemblages produced by later shell-gravel taxa occupying the same volume of sediment as earlier soft-bottom taxa; types III and IV beds should exhibit the opposite trend. These shifts in faunal composition should arise regardless of whether the dead hardparts that bring about the change in benthic habitat were produced *in situ* or delivered from allochthonous sources^{9,10}.

Another expected biotic response to variation in shell-packing density is a shift in average shell shape of morphologically plastic taxa—most notably epifauna—which can occupy both soft and shelly bottoms¹⁰. Shell gravel morphs should occur most frequently in assemblages from the most densely packed part of a shell bed (Table 1). Apparent morphometric variance within sampled fossil populations of species should also be greatest there, owing to the time-averaging of the directional shift from soft-bottom and shell gravel morphs and of random non-directional fluctuations in morphology. In types III and IV shell beds, the expected burst of variation followed by a gradual dwindling of variance mimics patterns predicted by some theories of speciation^{11,12}.

The robustness of the sedimentological model is evaluated by relaxing the assumptions, using a series of hypothetical histories of sedimentation and hardpart input (Fig. 2). When hardpart input is allowed to vary (Fig. 2b-e), classification and interpretation of shell beds using the bed contact criterion yields a correct interpretation of sedimentary dynamics in all but one situation: when peaks in hardpart input coincide precisely with maxima in sedimentation (Fig. 2d). This relationship is biologically improbable, because most shelly benthos avoid colonizing settings with high rates of sedimentation. Coincident maxima can occur when hardparts are hydraulically equivalent to enclosing sediments, such as macroinvertebrate shell debris in turbidity current or other high-energy conditions, and microfossil tests in deep-sea environments. These pitfalls can be diagnosed in the field by visual comparison of the hardpart and sediment grain sizes. Figure 2e,f illustrates the results of more complicated patterns of change in sedimentation and hardpart input and more closely mimics real fossiliferous sequences. The robustness of the model for these, as well as for endmember conditions, suggests that the model can be applied successfully to the spectrum of possible combinations of rate changes.

Changes in net sedimentation inferred from bed contacts provide working hypotheses that can be tested by their palaeontological predictions (Table 1) as well as by independent evidence for reduced net sedimentation within the shell bed (glauconite concentration, winnowed sedimentary matrix, physically amalgamated beds). In the Maryland Miocene sequence,

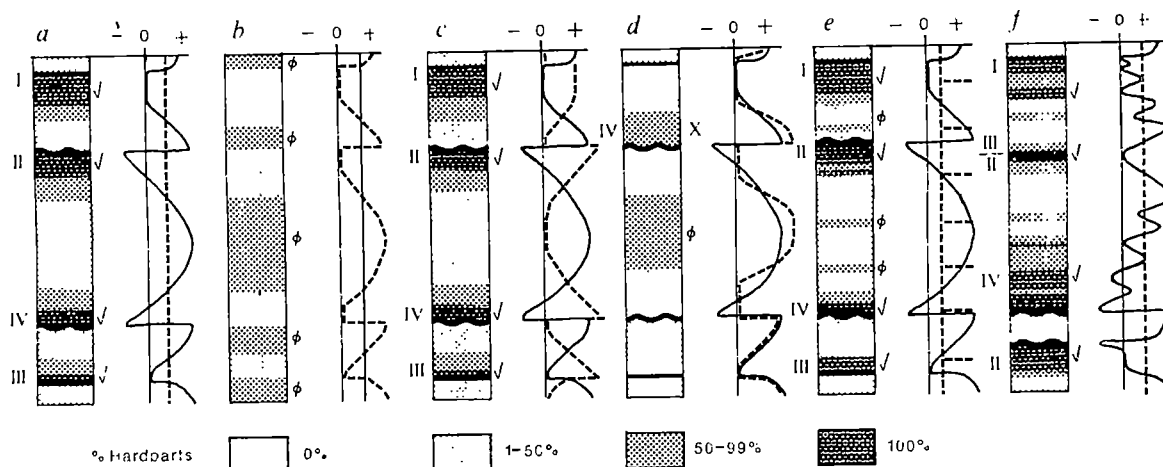


Fig. 2 Synthetic fossiliferous sequences generated from hypothetical patterns of change in sedimentation (solid curve) and hardpart input (dashed curve) provide a test of the robustness of the simple sedimentation model of fossil concentration (Fig. 1). *a*, Condition of constant hardpart input (original model conditions). The sedimentation model correctly interprets the mode of formation of all hardpart concentrations that can be classified as type I, II, III or IV (indicated by ✓). *b*, Constant sedimentation, only hardpart input varies. Concentrations form when hardpart input is relatively high, but none is associated with sharp discontinuity surfaces that permit classification by the model, the model does not lead to erroneous interpretations of the mode of formation (indicated by φ). *c*, Hardpart input varies inversely with sedimentation. Variation in hardpart input simply accentuates concentrations produced by excursions in sedimentation, and thus the model correctly interprets shell beds. *d*, Hardpart input varies directly with sedimentation. When the two rates are identical, fossil abundance does not vary stratigraphically (lower part of synthetic section), but relative concentrations do arise when hardpart input is allowed to exceed sedimentation. The model yields incorrect interpretations (indicated by X) of only those shell beds actually associated with discontinuities. This requires a very particular pattern of sedimentation plus perfect hydraulic equivalence of hardparts with sediment or preferential colonization by macrobenthos during the episode of maximum sedimentation. *e*, Variable net sedimentation, constant background rate of hardpart input with brief positive excursions (for example, mass mortality, allochthonous input). Synthetic pattern of fossil abundance closely mimics real stratigraphical sequences wherein some concentrations are associated with stratigraphical discontinuities, concentrations that can be classified by bed contacts are correctly interpreted by the simple sedimentation model despite random variation in hardpart input. *f*, Complex changes in sedimentation plotted against constant hardpart input. Short-term fluctuations during overall decrease or increase in net sedimentation produce internally complex fossil concentrations; back-to-back monotonic changes produce composite types I-III and II-IV deposits in which both upper and lower contacts are sharp; the model is robust to any pattern of change in sedimentation.

all four large-scale shell beds (Beds 10, 14, 17 and 19 (ref. 13) in Fig. 3a) and most smaller-scale shell concentrations can be classified on the basis of bed contacts as type I, II, III or IV. These exhibit physical evidence of accumulation during intervals of reduced net sedimentation (letter-coded in Fig. 3). Expected palaeontological trends are best developed in the large-scale accumulations, which record skeletal accumulation over prolonged periods of time (estimated at 10^{-3} – 10^{-4} yr each³). As evident in the expanded columnar sections of beds 10 and 17 (Fig. 3b, c), these shell beds exhibit similar vertical sequences: a basal shell hash of largely fragmental infaunal and unbroken epifaunal hardparts; this grades into the main body of the shell bed which contains ecologically mixed and highly amalgamated assemblages of both whole and broken closely packed shells; and an upper interval of closely spaced but discrete shell horizons by which the shell bed grades into less fossiliferous overlying strata. The small-scale types I and IV shell beds which characterize the upper transitional part of these thick, complex type IV shell beds are lags of reworked hardparts from soft-bottom communities, and in some instances have been colonized by epifauna⁹. Directional shifts in bivalve morphology documented within each of the major Miocene shell beds have been interpreted as microevolutionary in origin¹⁴, but might also represent the predicted ecological response to changing substratum characteristics or to concomitant increases in water depth deepening during transgression, or the expected effects of time-averaging. Expected palaeontological trends are thus borne out on several hierarchical scales of skeletal accumulation in this particular setting⁴.

These results with both synthetic and real stratigraphical sections suggest that the model has more than heuristic value. The model should promote palaeobiological comparison of fossil assemblages because it can be used as a testable hypothesis to address a wide variety of skeletal concentrations. Hardpart accumulations can be evaluated regardless of their timescale of

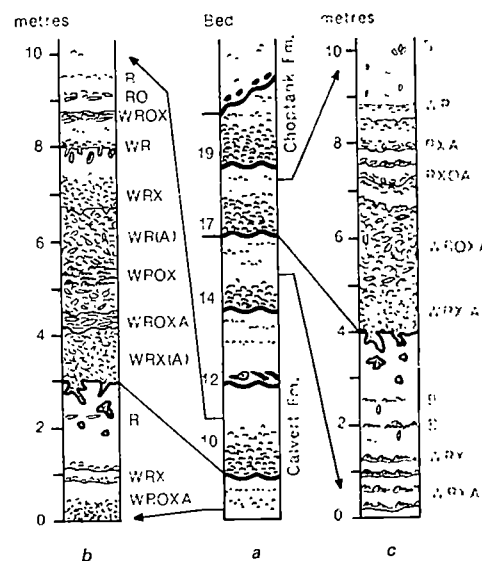


Fig. 3 The Middle Miocene Calvert and Choptank formations (composite section, middle column *a*) include four thick (1–10 m) and laterally extensive (2,500–7,000 km²) shell beds (numbered 10, 14, 17 and 19 in column *a*) and many small-scale concentrations. Most of these are associated with stratigraphical discontinuities—disconformities in the case of the four major shell beds, and scoured or winnowed bedding planes in the case of the minor accumulations (see schematic columns *b* and *c* for expanded scales)—and exhibit independent evidence of concentration during low net rates of sedimentation¹⁵. W, Winnowed matrix; R, hydraulically reoriented specimens; O, overpacked infauna; A, physically amalgamated beds; X, exhumed specimens; B, concentrations of biogenic origin, for example, clumps of shells recording concentration through biological processes, such as gregarious behaviour of the skeletalized fauna or reworking by other organisms.

accumulation (for example, both rapidly formed storm beds and slowly accumulated basal lags on unconformities have type IV structures), physical dimensions, taxonomic composition, proportion of allochthonous hardparts, depositional environment and geological age. Detailed palaeoecological and evolutionary comparisons across environmental gradients or through geological time thus become feasible if a single type of shell bed is sampled throughout. For example, a transect based on erosive washover deposits, swash zone shell laminae, lags produced by channel and shoal migration, inner shelf storm concentrations and shelly proximal turbidites—which are all short-term type IV deposits—should yield assemblages that are ecologically and taphonomically more comparable with one another than to assemblages from short-term types I, II or III deposits. In addition, the absence (or otherwise divergence) of taphonomic trends from those predicted by the model permit the detection of more complex histories and conditions of fossil concentration. Successive horizons within a shell bed might be palaeontologically identical because of: very rapid concentration and burial which sequesters hardparts from destructive processes at the sea floor so that trends never develop; accumulation in environmental conditions (for example anoxia) which exclude potential colonists of dead hardparts; and homogenization by bioturbation. Very rapid formation and burial relative to rates of hardpart destruction and benthic colonization probably explain the less consistent development of expected trends within the small-scale shell beds of the Maryland Miocene, and bioturbation of shell bed contacts may well obscure the sedimentological origin of many others. The complex internal stratigraphies of the major shell beds, on the other hand, record the physical amalgamation of many small-scale concentrations into a single fossil deposit by the superposition of short-term fluctuations on a longer, overall trend in sedimentation rate, such as examined synthetically in Fig. 2f.

If sedimentation is the primary control on skeletal accumulation as indicated by the initial tests reported here, fossil concentrations can yield insights into the basic dynamics (timing and magnitude) of stratigraphical accumulation. The relative frequency of type I, II, III and IV beds should vary among depositional environments owing to differences in sediment supply and hydrodynamics, and suggests a new approach to the mapping and interpretation of sedimentary facies. Qualitatively different patterns of fossil accumulation can also be expected between transgressive and regressive phases of marine deposition, and among basins of different tectonic and latitudinal settings. By referring to fundamental rates and patterns of hardpart input and sedimentation, this approach to modelling has the potential to generate testable hypotheses for a systematic exploration of the nature of the fossil record on several scales.

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Sex ratios of an aphid subject to local mate competition with variable maternal condition

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Fisher¹ was the first to argue that natural selection would adjust the sex ratio so as to equalize parental investment (PI) in the two sexes, where mating is at random. Hamilton² then showed that female-biased sex ratios would be favoured where male siblings compete for matings, a situation referred to as 'local mate competition' (LMC). In Hamilton's original model and in most subsequent LMC models, the females founding a local breeding population have equal amounts of PI to be allocated between sons and daughters. But in nature, females may differ in total PI and, as a consequence, in fecundity. Here I describe a model for the sex ratios of n co-foundresses, all of which differ in total PI. The model shows that each female with more than a specified minimum amount of PI is selected to make the same absolute investment in sons. All previously published cases of dramatic sex-ratio control occur in haplodiploid species. Here I test my theoretical model against sex-ratio data for an aphid, *Prociphilus oris*, which has a normal diploid genetic system.

Several theorists have considered the sex-ratio strategies of two co-foundresses with different clutch sizes, and they have found that the more fecund female should always produce the more female-biased ratio of investment^{3,4}. 'Double parasitism' of hosts often occurs in natural populations of parasitoid wasps, leading to local competition for mates among the offspring of two females. But in aphids, such LMC often occurs among the offspring of more than two females. Thus for aphids, a more general LMC model is needed.

The model described here embodies the following assumptions. Each local breeding population ('patch') is founded by n inseminated females selected at random from the total population at large. These females differ in their total PI: $P_1 < P_2 < \dots < P_n$, where P_i is the total PI of the i th female. Each female can control her offspring sex ratio, S_i , which is the proportion of P_i invested in sons. Mating occurs at random among the offspring born in the same patch, and all of the female offspring are successfully inseminated. Then the mated female offspring disperse, becoming thoroughly mixed with the rest of the population at large before the next generation's patches are established by taking samples of n females.

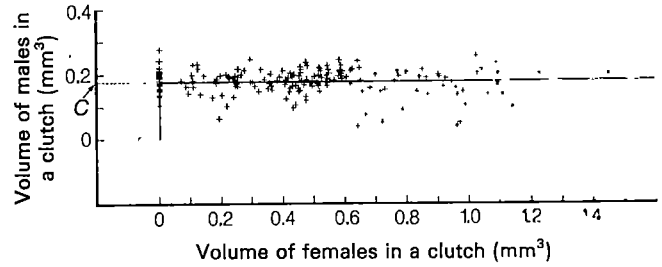
Natural selection will favour offspring sex ratios that maximize each mother's inclusive fitness. With a diploid genetic system and autosomal control of the sex ratio, mothers are related equally to their sons and daughters. At the end of the period of parental investment, the expected reproductive success of female offspring (R_f) can be taken to be a constant, equal for all daughters of all mothers. But the reproductive success of a son (R_m) depends directly and immediately on the sex ratios of the mothers in the patch. Thus the inclusive fitness of each mother is a function of her own sex ratio and of the sex ratios of the other mothers, and sex-ratio evolution takes the form of a game in which the best move for each individual depends on what all the others are doing⁵. Below, I derive the optimal sex ratios as the Nash solution or non-cooperative equilibrium of an n -person game, which in this context is the same as an evolutionarily stable strategy⁶.

The inclusive fitness of the i th mother (F_i) in a patch founded by n mothers can be written as⁷

$$F_i = r_d \frac{P_i(1-S_i)}{w_d} R_f + r_s \frac{P_i S_i}{w_s} R_m \quad (i = 1, 2, \dots, n) \quad (1)$$

where S_i = sex ratio of the i th mother; P_i = total PI of the i th mother ($P_1 < P_2 < \dots < P_n$); r_d , r_s = relatedness of a mother to

Fig. 1 Parental investment in sons and daughters, for 176 sexuparae of the aphid *P. oriens*. Total PI in male offspring is indicated on the ordinate ($P_i S_i$), with PI in female offspring shown on the abscissa; $P_i(1-S_i)$. Twenty sexuparae produced sons exclusively; these are represented by the vertical column of points at the left-hand end of the scattergram. Each sexupara produced at least one son. The superimposed rules show the distribution of investments predicted by the theoretical model described in the text.



daughters and sons, respectively; R_f , R_m = reproductive success of a daughter or a son, respectively; w_d , w_s = cost of a daughter or a son, respectively, in units of PI.

Assume that $r_d = r_s$ and that R_f is a constant. It follows that

$$R_m = \frac{\sum_{i=1}^n P_i(1-S_i)/w_d}{\sum_{i=1}^n P_i S_i/w_s} R_f \quad (2)$$

From equations (1) and (2), we have

$$\frac{\partial F_i}{\partial S_i} \propto -1 + \frac{\sum_{i=1}^n P_i(1-S_i)}{\sum_{i=1}^n P_i S_i} - \frac{\sum_{i=1}^n P_i}{(\sum_{i=1}^n P_i S_i)^2} P_i S_i \quad (3)$$

The mean sex ratio is defined as

$$\bar{S} = \frac{\sum_{i=1}^n P_i S_i^*}{\sum_{i=1}^n P_i} \quad (4)$$

where S_i^* is the optimal sex ratio of the i th mother. The Nash equilibrium conditions are

$$(\partial F_i / \partial S_i)_{S_i=S_i^*} \leq 0 \quad \text{if} \quad S_i^* = 0 \quad (5a)$$

$$(\partial F_i / \partial S_i)_{S_i=S_i^*} = 0 \quad \text{if} \quad 0 < S_i^* < 1 \quad (5b)$$

$$(\partial F_i / \partial S_i)_{S_i=S_i^*} \geq 0 \quad \text{if} \quad S_i^* = 1 \quad (5c)$$

By using equation (3), equations (5a) to (5c) can be rewritten as

$$P_i S_i^* \geq \bar{S}(1-2\bar{S}) \sum_{i=1}^n P_i \quad \text{if} \quad S_i^* = 0 \quad (6a)$$

$$P_i S_i^* = \bar{S}(1-2\bar{S}) \sum_{i=1}^n P_i \quad \text{if} \quad 0 < S_i^* < 1 \quad (6b)$$

$$P_i S_i^* \leq \bar{S}(1-2\bar{S}) \sum_{i=1}^n P_i \quad \text{if} \quad S_i^* = 1 \quad (6c)$$

It is convenient to define C as

$$C = \bar{S}(1-2\bar{S}) \sum_{i=1}^n P_i \quad (7)$$

Then equations (6a) to (6c) immediately simplify to

$$0 \geq C \quad \text{if} \quad S_i^* = 0 \quad (8a)$$

$$0 < P_i S_i^* = C \quad \text{if} \quad 0 < S_i^* < 1 \quad (8b)$$

$$0 < P_i \leq C \quad \text{if} \quad S_i^* = 1 \quad (8c)$$

I now consider where each of equations (8a) to (8c) will hold:
Case 1. $S_i^* = 0$. This is impossible. If even one mother produces any sons ($S_i > 0$), then $C > 0$ and equation (8a) is contradicted.

Case 2. $0 < S_i^* < 1$. Equation (8b) can be rearranged to give

$$S_i^* = C/P_i \quad (9)$$

and the condition $S_i^* < 1$ can be rewritten as

$$P_i < C \quad (i = 1, 2, \dots, n) \quad (10)$$

Case 3. $S_i^* = 1$. Equation (8c) implies that

$$P_i \leq C \quad (11)$$

The assumption that all mothers produce $S_i^* = 1$ leads to a contradiction, but a mixture of sex ratios from case 3 ($S_i^* = 1$) and case 2 ($0 < S_i^* < 1$) is possible, because equations (8b) and

(8c) can both be satisfied when $0 < \bar{S} < 0.5$. Thus the Nash equilibrium is

$$S_i^* = C/P_i \quad \text{for} \quad P_i > C \quad (12a)$$

$$S_i^* = 1 \quad \text{for} \quad P_i \leq C \quad (12b)$$

Next, I show that the Nash equilibrium is determined uniquely if the P_i values are given. Substituting equations (12a) and (12b) in equation (4), we obtain

$$\begin{aligned} \bar{S} &= \frac{\sum_{i=1}^n \min(P_i, C)}{\sum_{i=1}^n P_i} \\ &= \left\{ \sum_{i=1}^m P_i + C(n-m) \right\} / \sum_{i=1}^n P_i \end{aligned} \quad (13)$$

where it is assumed that the P_i values are ordered as an increasing series. Equation (13) shows that C is a semi-concave function of \bar{S} , while equation (7) shows that C is a convex function of \bar{S} ($0 < \bar{S} < 0.5$). Thus, there is a unique solution (\bar{S}, C) that satisfies both equations (7) and (13), and m is uniquely determined ($P_m \leq C < P_{m+1}$).

Finally, I calculate \bar{S} . If all mothers produce sex ratios strictly between 0 and 1 (case 2), we have an instance of the 'special case', while if some mothers produce sex ratios of unity (case 3) we have an instance of the 'general case'. The special case represents the general case with $m = 0$.

General case.

$$S_i^* = 1 \quad (i = 1, 2, \dots, m)$$

$$S_i^* = C/P_i \quad (i = m+1, \dots, n) \quad (14)$$

Equation (7) together with equation (13) gives

$$\bar{S} = \frac{1}{4(n-m)} \left\{ (n-m-1) + \sqrt{(n-m-1)^2 + 8(n-m) \frac{\sum_{i=1}^m P_i}{\sum_{i=1}^n P_i}} \right\} \quad (15)$$

C is determined by substituting equation (15) in equation (7).
Special case.

$$S_i^* = C/P_i \quad (i = 1, 2, \dots, n) \quad (16)$$

Combining equation (6b) with equation (4) yields

$$\bar{S} = \frac{1}{2}(n-1)/n \quad (17)$$

Substituting equation (17) in equation (7), we obtain

$$C = \left(\sum_{i=1}^n P_i / n \right) \{ (n-1)/2n \} \quad (18)$$

The equilibrium sex ratios are uniquely determined by the P_i . The optimal sex ratio of the i th mother (S_i^*) is inversely related to her total PI (P_i), if P_i is larger than the constant C ; see equations (14) and (16). This result generalizes the existing sex-ratio models for two foundresses with unequal PI. But the most surprising result is that $C = P_i S_i^*$ —in other words, all mothers with more than C units of total PI are selected to make the same absolute investment in sons, while mothers with less than C units of PI are selected to produce sons exclusively. This is a new result.

If there is little variation in total PI, such that all mothers make some daughters (special case), then the average sex ratio

in each patch is $\bar{S} = \frac{1}{2}(n-1)/n$; see equation (17). This is the same as Hamilton's equilibrium sex ratio for the case in which all females have the same PI. But if some mothers have less than C units of PI (and therefore produce sons exclusively; general case), Hamilton's expectation does not give the exact average patch sex ratio; see equation (15).

At equilibrium, the effect of sibling competition on the inclusive fitness of each mother is the same, because each female produces the same number of sons. This would not be true if, for example, each female produced the same offspring sex ratio, regardless of her total PI.

The aphid *Prociphilus oriens* (Homoptera, Pemphigidae) is a useful species with which to test sex-ratio theories, owing to several features of its biology. It undergoes sexual reproduction once a year, in the autumn. The sexuparae ('mothers', for purposes of testing this model) produce sexuales (males and oviparous females) parthenogenetically. The sexuparae mature and feed on the secondary host, *Abies sachalinensis*. During this period, embryonic sexuales are developing within the sexuparae. When they have finished feeding, the sexuparae moult and fly to the primary host, *Fraxinus mandshurica*. They move around on the primary host for a few hours, looking for places to deposit their offspring; this wandering gives rise to a patchy distribution of sexuparae on the bark of the primary host. Within half a day the sexuparae deposit their offspring and die.

The sexuales stay at their place of birth for a few days, and then become sexually active. The males have very limited powers of movement, and they are sexually active for only about one day. Thus, the sexuales produced by the mothers in a given patch are effectively a breeding population within which there is likely to be significant local mate competition. Mothers appear to have direct control over the sex ratios of their offspring⁸ and their fecundities over a wide range. The observations of mating behaviour of the sexuales showed that they cannot recognize siblings. Thus, *P. oriens* seems to satisfy all of the assumptions of the model.

Sexuales of both sexes complete their development within the bodies of the sexuparae. Lacking mouthparts, they do not feed after birth. Thus the volume of each offspring is an excellent index of its mother's investment in it⁹. The sexuales of *P. oriens* are remarkably dimorphic at birth. The average volume of a male is 0.047 mm³, while that of a female is 0.126 mm³.

Sexuparae were sampled at a site on Hokkaido, the northern island of Japan, in 1982. Sexuparae that flew to a clump of *F. mandshurica* during a period of several hours were caught as they attempted to land on the trees. They were fixed in 75% ethanol and later dissected. For each sexupara I determined the number of her male and female sexuales, and I estimated the volume of each individual sexuelle. The total PI of each sexupara (P_i) was defined as the total volume of her clutch, and her PI ratio (S_i) was then defined as the proportion of the total volume that consisted of sons.

For the sample of 176 sexuparae, the overall numerical sex ratio was almost exactly 0.5 males, while the overall PI ratio was 0.37, which is strongly and significantly female-biased. The difference between the numerical ratio and the PI ratio is caused by the sexual dimorphism of body size mentioned above.

Figure 1 shows the observed PI in sons and daughters for each sexupara. Superimposed on the data are lines illustrating the distribution of PI values predicted by the model, using the value of C calculated from the data. Mothers with less than C mm³ of total PI should invest exclusively in sons (the vertical line), while mothers with over C mm³ should invest C mm³ in sons and the rest in daughters (the horizontal line). The data cluster remarkably well about the theoretical lines.

If females could have been sampled after they had aggregated into local patches, C could have been determined directly from the model, using the observed average value of n and the observed distributions of P_i within patches. Because this was not feasible, I calculated C as the population average of the total volume of males in a clutch. This C value is equivalent to $\bar{C} = \sum_{j=1}^K C_j/K$ ($j=1, 2, \dots, K$, where K is the number of

patches), because the total number of sexuparae sampled would divide into many patches. The good agreement of this C value with the data suggests that the variation of C between the patches is small. The observed mean investment ratio ($\bar{S} = 0.37$) implies that a typical patch contains the offspring of approximately four sexuparae; equations (15) and (17). This inferred value of n is fully consistent with my observations of the behaviour of *P. oriens* at the site from which the sexuparae were obtained. The PI distribution within a patch, however, may vary between patches in nature. Constructing a model which includes the variation of total PI between the patches and also that of the patch size, n , is a job for the future.

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Age-impaired impulse flow from nucleus basalis to cortex

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Recent studies have renewed interest in the role of acetylcholine (ACh) in the cognitive changes associated with ageing and dementia¹⁻⁴. Deficits in cortical choline acetyltransferase (ChAT) in Alzheimer's disease have been consistently demonstrated⁵⁻⁹, while other research has suggested a connection between deterioration of cortical ACh fibres and dementia⁴. However, despite clear biochemical and anatomical evidence for a fall in ACh in dementia¹⁻⁹, results of therapeutic trials with cholinergic agonists, precursors and cholinesterase inhibitors have been inconsistent⁶⁻⁹. Such findings suggest that cortical cholinergic disorders are not wholly a function of simple biochemical change; alterations of impulse flow along cholinergic fibres could well be as debilitating. An important extrinsic source of cortical ACh innervation derives from neurones diffusely located in rat basal forebrain¹⁰⁻¹⁶, denoted the nucleus basalis (NB)^{15,17}. We have now investigated the impulse conduction properties of cortically projecting, putatively cholinergic NB axons in adult and aged rats and have found that conduction latencies from NB to frontal cortex are significantly longer (by 51%) in aged animals. In addition, systematic analysis varying cortical stimulation depth revealed that these longer latencies are due entirely to decreased conduction velocities in the subcortical fibre projections. Indeed, intracortical velocities were virtually identical in the two groups. Our results indicate that ageing occasions a decrease in the temporal fidelity of impulse flow in the cholinergic input to the cortex from the NB, a previously overlooked but potentially important element in cognitive deficits that occur with age.

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Fig. 1 Photomicrograph of a coronal section through the basal forebrain of an aged experimental rat (neutral red stain). Pontamine sky blue (spots in NB area, at arrows) was iontophoresed at the locations of two cells driven antidromically from prefrontal cortex. All cells in this study were localized histologically to the NB area in this manner. For calibration, Pontamine spots are 500 μm apart. GP, globus pallidus; IC, internal capsule; CP, caudate putamen.

Six adult (6–8 months) and seven aged (20–29 months) female Sprague-Dawley rats were anaesthetized with chloral hydrate throughout experimental sessions. Aged rats were in good health and remained as viable as adult rats under anaesthesia. A twisted pair of 250- μm -diameter wires, insulated except for bluntly cut tips, was used to stimulate the prefrontal cortex, while a glass micropipette served to record impulses from single NB neurones. The stimulation electrode was placed 0.5 mm below the cortical surface and then raised or lowered in 0.3-mm steps when testing individual cells. Details of stimulation and recording procedures are published elsewhere^{18,19}. Recording sites were marked at the end of successful penetrations by iontophoresing dye from pipette tips. All cells reported here were histologically localized to the NB area (Fig. 1), corresponding to previous anatomical maps of cortically projecting acetylcholinesterase or ChAT-positive neurones^{13,15,20,21}. To control for possible effects of decreased ovarian function with age, half the control adults were ovariectomized 4–6 weeks before experiments. No difference was seen between ovariectomized and intact control rats, and these results were pooled.

Of more than 400 neurones recorded in the vicinity of the NB, 35 in aged animals and 25 in young animals were activated antidromically from the prefrontal cortex. Criteria for antidromicity included constant latency driving, high-frequency activation and collision between spontaneous and driven impulses^{18,19} (Fig. 2). These cells exhibited a variety of rates and patterns of spontaneous activity, resembling the physiological heterogeneity previously found for cortically projecting NB neurones^{18,19}. As also reported previously for young adult male rats^{18,19}, conduction latencies varied widely, yielding values ranging from 1.2 to 16.0 ms in young controls. Despite this heterogeneity, latencies in aged rats were longer overall than in controls, ranging from 2.1 to 27 ms. The mean latency for aged subjects (8.6 ± 0.5 ms) was significantly greater than that for the young group (5.7 ± 0.3 ms), exhibiting a 51% increase ($F = 21.1$, $P < 0.001$).

Nearly every NB neurone, in both young and old rats, could be activated antidromically from the entire depth of the cortex, thus enabling us to compare intracortical and subcortical aspects of the age change in NB axon conduction velocity. Antidromic latencies decreased significantly in a linear fashion overall as the stimulating electrode was lowered to deeper cortical sites ($F = 5.80$, $P < 0.02$) (Fig. 3a). However, although conduction latencies for old rats were consistently longer than for young rats at all cortical depths, slopes of the curves were virtually identical for both groups, yielding estimated intracortical conduction velocities of 0.8 m s^{-1} regardless of age (Fig. 3a). Thus, the entire difference in conduction times between the two groups

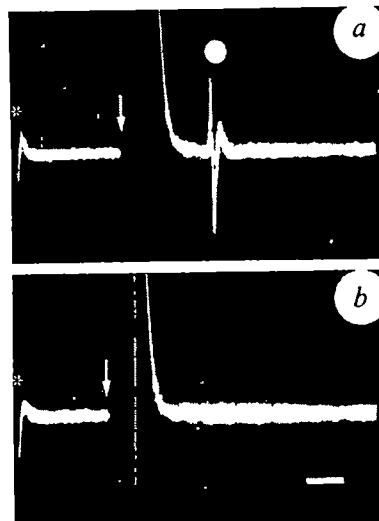


Fig. 2 Oscilloscope photographs illustrating collision test for an NB neurone driven from prefrontal cortex in an aged rat. *a*, Stimulation of cortex (arrow) 12 ms after spontaneous spikes (asterisk) elicits driven spikes (filled circle) at 10 ms latency. *b*, Driven spikes are occluded for similar stimuli (arrow) delivered 11 ms after spontaneous spikes (asterisk), indicating collision between spontaneous and driven impulses. Horizontal calibration, 5 ms. Ten superimposed sweeps in each trace.

is accounted for by significantly slower subcortical conduction velocities in aged rats (Fig. 3b).

In contrast to the above conduction changes, several other physiological parameters of NB neurones remained stable in old age. (1) Absolute refractory periods averaged 2.6 ± 0.3 ms in adult controls and 2.4 ± 0.2 ms in aged rats. (2) The number of cells that could be activated antidromically averaged 0.8 ± 0.2 cells per penetration for both age groups. (3) The propensity of axons to exhibit multiple discrete antidromic latencies, previously suggested to reflect intracortical branching¹⁹, was equally evident in both age groups, with averages of 2.8 ± 0.4 and 2.4 ± 0.3 discrete latencies per cell in young and old animals, respectively. (4) Thresholds for antidromic activation were not significantly different between age groups across cortical stimulation depths ($P > 0.1$, Student's *t*-test). (5) There was no apparent difference in the amplitudes of driven impulses in young and aged rats.

Several mechanisms may underlie these age-dependent decreases in NB conduction velocity. (1) Selective loss of larger NB axons, perhaps by shrinkage²², could produce such an effect. However, this process would seem likely also to alter stimulation thresholds, refractory periods and intracortical conduction velocities, none of which showed any significant change with age. Also, the distribution of antidromic latencies revealed an overall shift to longer latencies with age, rather than a loss of one subpopulation. These results, together with the finding that the frequency of encountering driven cells does not change with age, indicate that fibre shrinkage or selective cell loss in aged subjects is unlikely to account for our findings. (2) Similar reasoning argues against the possible mediation of these changes in conduction velocity by altered biophysical properties of the NB axolemma. Although age-associated changes such as increased membrane resistance and viscosity have been reported for other systems^{23,24}, such mechanisms would again be expected to alter not only conduction velocity, but also other electrophysiological characteristics. (3) A third possibility, more consistent with the present data, is demyelination of NB axons. The subcortical conduction velocities obtained for young animals (most of which were $> 3.0 \text{ m s}^{-1}$) indicate that NB axons are normally myelinated subcortically. However, conduction velocities for NB axons within cortex are significantly slower, with mean values (0.8 m s^{-1}) more suggestive of non-myelinated axons^{25,26}. Similar results have been obtained for adult male rats¹⁹. Thus, like mossy fibre afferents to the cerebellar cortex²⁷, NB fibres

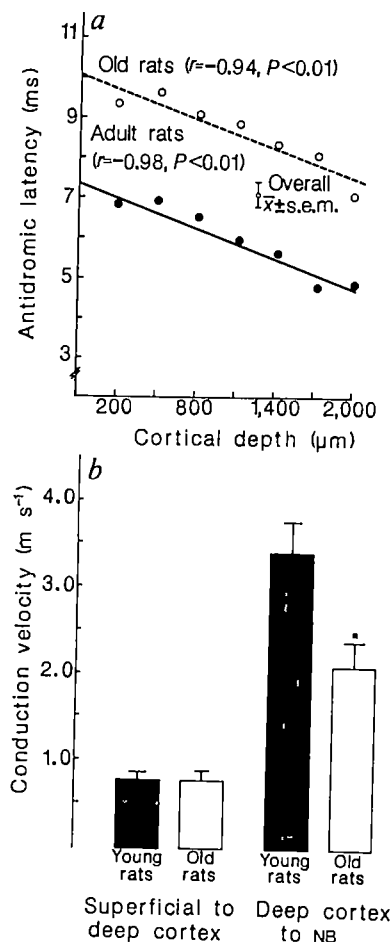


Fig. 3 *a*, Mean antidromic conduction latency for NB neurones in adult (filled circles) and aged (open circles) subjects after threshold stimulation at various depths in the prefrontal cortex, as indicated on the abscissa. Note that although conduction latencies are longer at all depths in aged rats, the slopes of the curves are virtually identical, indicating similar conduction times in the two age groups for intracortical impulse flow. In repeated measures analysis of variance, the interaction of age group with cortical depth was nearly zero, indicating that the effect of age was consistent at all cortical depths. The high correlation coefficient (r values) obtained in the regression analysis indicates that these fibres have a strong radial component in their intracortical trajectories, supporting previous anatomical studies^{11,12,36-38}. *b*, Mean conduction velocities for NB cortical afferent fibres in adult (filled bars) and aged (open bars) rats. Conduction velocities within the cortex were calculated for each cell and then averaged, using the difference between the latencies at threshold intensities for 200- and 2,000-μm stimulation depths and a corresponding distance of 1.8 mm. Subcortical conduction speeds (deep cortex to NB) were similarly calculated for each cell using the threshold latency at 2,000 μm and an estimated distance from NB to cortex of 10 mm. Note that conduction velocities within the cortex are virtually identical for adult and aged subjects. Subcortical conduction velocities for young rats (mean \pm s.e.m. = 3.4 ± 0.4 m s⁻¹) are over fourfold higher than those in the cortex, and show a significant decline with ageing (conduction velocity for aged rats = 2.1 ± 0.3 m s⁻¹; $t_{38} = 2.43, P = 0.02$).

may be myelinated until they enter the cortical grey matter¹⁹. Given such a subcortically myelinated projection system, the hypothesis of age-related demyelination is well in accord with all aspects of our data. It explains, for example, why intracortical conduction velocities remain stable with age while subcortical velocities decline markedly. Similarly, demyelination subcortically would not require alteration in refractory periods or thresholds within the cortex. Finally, such a mechanism would leave the relative numbers of fibres and cells intact, a point consistent with our results.

Further research, particularly at ultrastructural levels, will be necessary to test our hypothesis. We note, however, that de-

myelination, whether as a neuropathy in itself or as an effect secondary to axonal pathology, is an established basis for neurological disorders, occurring, for example, in Charcot-Marie-Tooth disease and Friederich's ataxia²⁸.

Ours is not the first report of altered fibre conduction velocity in senescence. Decreased impulse speeds have been found in human peripheral²⁹⁻³¹ and spinal nerves³¹, as well as rodent motoneurons²³ and cerebellar parallel fibres³². However, examples where there is no senescent change in fibre conduction velocity have been reported in both central^{33,34} and peripheral³⁵ nervous systems. Thus, decreased impulse conduction velocity in old age occurs in a restricted set of fibre systems. This selective action may underlie distinct symptoms of the overall ageing syndrome. For example, diminished cerebellar parallel fibre conduction velocity may impair motor coordination³², a common problem in ageing. Similarly, reduced temporal fidelity in the NB cortical afferent system, as described here, may promote cognitive deficiencies characteristic of senescence, and especially pronounced in dementia of the Alzheimer's type.

Although many behavioural studies of ageing have proposed a relationship between cognitive deficits and changes in cholinergic systems⁶⁻⁹, evidence for the latter at the biochemical level is scant. Cortical ChAT decline, for example, occurs consistently in Alzheimer's disease, but not in normal ageing¹⁹. This seldom-addressed paradox may be explained by our findings. Demyelination of cortically projecting cholinergic fibres with age would not necessarily require cholinergic biochemical concomitants, but would nonetheless impair the temporal fidelity of NB-cortical processing, thereby disrupting normal cognitive mechanisms.

If the primary function of the nervous system is to process information by virtue of electrical impulse flow, physiological measurements such as those in the present study may provide a relevant and sensitive index of geriatric cognitive decline.

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A late-differentiation antigen associated with the helper inducer function of human T cells

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T lymphocytes possessing helper function produce soluble factors that greatly augment B-cell proliferation and differentiation into antibody-secreting cells¹. In humans the subset of T lymphocytes bearing the T4 surface antigen comprises most of the cells that display helper activity and recognize class II antigens of the major histocompatibility complex (MHC), while the subset bearing the T8 antigen comprises T cells recognizing class I MHC antigens and exhibiting cytotoxic or suppressor function¹⁻³. Monoclonal antibodies to T4 or T8 greatly inhibit the cognitive and effector function of cells with the corresponding phenotype¹⁻⁴. This function/phenotype correlation is not absolute, however, for there are many examples of T8-positive clones that recognize MHC class II antigens and have helper activity, as well as of T4-positive clones with suppressor or cytotoxic function⁵⁻⁹. Recently a family of cell-surface neoantigens, which might be relevant to T-cell function and which are present on activated but not on resting T lymphocytes, has been identified in mouse and humans using monoclonal antibodies¹⁰. Some of these antibodies block the cytolytic activity of alloreactive T-cell clones, suggesting the possible involvement of such molecules in the activation of cytotoxic T-cell clones or in the lytic process itself¹⁰⁻¹³. We now describe a similar late-differentiation antigen (LDA₁) that is expressed by human T lymphocytes only following activation and is recognized by a monoclonal antibody that inhibits the antibody-inducing helper function of T lymphocytes.

Monoclonal antibody anti-LDA₁ was produced by immunizing a BALB/c mouse with an alloreactive T-cell clone (TCC 19) possessing helper function^{14,15}. This hybridoma was originally selected because it reacted with the immunizing T-cell clone (no. 19) but not with peripheral blood T or B lymphocytes or with Epstein-Barr virus-transformed B-lymphoblastoid cell lines from the same donor or from 30 unrelated individuals. Further screening of this antibody on alloresponsive T-cell clones showed that it reacted with all of the 24 helper but with none of the 14 cytotoxic T-cell clones tested. This antibody is of IgG1 subclass as determined by standard immunodiffusion procedures, and was used as an optimally diluted ascitic fluid in most experiments.

Indirect immunofluorescence using an Ortho Spectrum III cytofluorograph^{14,15} showed that LDA₁ is absent on resting T lymphocytes, but appears 1-3 days after the peak of DNA synthesis on a fraction of T lymphoblasts stimulated *in vitro* with plant mitogens, soluble antigens or allogeneic cells. The expression of LDA₁ is dependent on cell proliferation and protein synthesis, as it is prevented by treatment of cells with mitomycin C or emetine-HCl shortly after activation¹⁶. Anti-LDA₁ had no effect on the blastogenic responses of fresh T lymphocytes to plant mitogens, soluble antigens [tetanus toxoid (TT) and purified protein derivative of tuberculin (PPD)] or allogeneic stimulating cells.

Cytofluorometric evaluation of LDA₁ expression on cells grown in primary mixed lymphocyte culture (MLC) showed that 30% LDA₁-positive T blasts are present by day 9, decreasing to <5% by day 14. The fraction of the population expressing LDA₁ was increased, however, to 70% when day-6 MLC-T lymphoblasts were expanded by continuous stimulation with irradiated cells from the sensitizing donor in medium supplemented with interleukin-2.

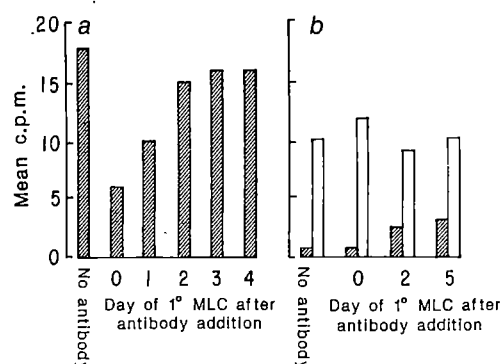


Fig. 1 Replicate cultures containing 10^7 PBM from an individual with the HLA-DR5,9 phenotype were stimulated in 20-ml tissue culture flasks with an equal number of irradiated PBM from an HLA-DR5,7 donor. Anti-LDA₁ was added at a final dilution of 1:100 to individual culture flasks on day 0, 1, 2, 3, 4 or 5. Control cultures contained no antibody. Ten days after the initiation of the primary cultures, cells were collected from each flask, centrifuged on a Ficoll gradient for removal of dead cell debris, adjusted to 5×10^4 viable responding cells per Microtest well and challenged in triplicate reactions with HLA-DR7-positive (■) or DR7-negative (□) stimulating cells. Secondary cultures were labelled with ^3H -thymidine for 18 h and collected on day 3 (a) or day 5 (b) following restimulation. Results are expressed as mean c.p.m. $\times 10^3$.

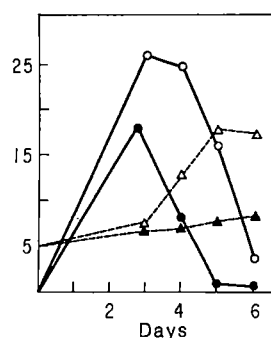


Fig. 2 The NSF-K T-cell line was developed by priming PBM from a DR2 homozygous individual to irradiated PBM from a DR3 homozygous stimulator. Day-6 MLC blasts were propagated for 28 days by weekly stimulation with DR3-positive cells in medium containing 20% interleukin-2. Responding T lymphoblasts (5×10^6 culture) were tested for reactivity to DR3-positive stimulating cells in the absence or presence of anti-LDA₁ (1:100 final dilution). The number of viable cells and the rate of ^3H -thymidine incorporation were determined on days 3-6. ○, c.p.m. $\times 10^3$ per 10^5 T cells grown without antibody; ●, c.p.m. $\times 10^3$ per 10^5 T cells grown with anti-LDA₁; △, number of cells ($\times 10^6$) in medium without antibody; ▲, number of cells ($\times 10^6$) in medium containing anti-LDA₁.

To determine whether the number of LDA₁-positive cells could be increased further, T-cell lines that had been propagated for 1 month *in vitro* and which expressed LDA₁ on 70% of the cells, were sorted into LDA₁-positive and -negative fractions using a fluorescence-activated cell sorter (FACS-I; Becton-Dickinson). Both the positively and negatively selected fractions exhibited $30 \pm 12\%$ LDA₁-positive T lymphoblasts after 48 h, and $60 \pm 15\%$ positive cells 120 h after sorting and expansion in cultures. Similar variations in LDA₁ expression were found on the immunizing TCC 19. This helper line, whose monoclonal origin was confirmed by studying the rearrangement of the T-cell antigen receptor β -chain¹⁷, displayed $40 \pm 18\%$ and $80 \pm 15\%$ LDA₁-positive cells, respectively, at 48 and 120 h following each feeding. This indicates that LDA₁ expression is modified on the surface of the alloactivated T lymphoblasts by which it is synthesized.

When added at the initiation of primary MLC cultures, the anti-LDA₁ antibodies did not significantly inhibit the primary day-5 blastogenic response, nor the generation of cytotoxic T

cells in primary MLC or the lytic activity of such cells as determined in a ^{51}Cr -release assay¹⁵.

Because the expression of LDA_1 is acquired only during late stages of the primary MLC response (day 9), we investigated the effect of anti- LDA_1 on secondary MLC reactions, which are usually tested on day 10. The secondary MLC response or the primed lymphocyte test detects accelerated memory responses displayed by cells that have been primed to a specific class II MHC antigen in primary cultures¹⁸. Non-activated cells from the same cultures do not participate in the secondary MLC response, yet maintain the ability to display primary blastogenic responses to the HLA-D/DR antigens for which they bear specific receptors.

Lymphocytes from a responder bearing the HLA-DR5,9 phenotype were primed in MLC to HLA-DR5, 7 stimulating cells. Anti- LDA_1 was added to individual cultures on day 0, 1, 2, 3, 4 or 5 and the secondary MLC response was tested on day 10 by rechallenging the cultures with stimulating cells. The reaction to specific, HLA-DR7-positive stimulating cells was an accelerated memory response, which peaked at day 3 and declined by day 5. The day-3 response was strongly inhibited when anti- LDA_1 was added to the cultures within the first 24 h of primary stimulation, but not when added later (Fig. 1a). None of the cultures showed accelerated (day 3) memory responses to HLA-DR7-negative stimulating cells. However, they displayed primary-type blastogenic responses to such cells on day 5, as expected for a polyclonal population of T cells that can recognize any allelic variants of (non-self) HLA-D/DR antigens in primary cultures. This primary anti-HLA-DR blastogenic response was not significantly inhibited in cultures grown with anti- LDA_1 (Fig. 1b). It appears, therefore, that anti- LDA_1 inhibits the capacity of primed T cells to respond to the sensitizing antigen, as further suggested by its suppressive effect on alloreactive T-cell lines (Fig. 2). The finding that LDA_1 is expressed by T lymphocytes only after activation may explain the difference between the effect of anti- LDA_1 on primary and secondary MLC.

Table 1 Effect of anti- LDA_1 on T-helper function

Individual	Antibody added	Immunoglobulin (ng ml ⁻¹) in cultures with					
		No antigen		Tetanus toxoid		PWM	
		IgG	IgM	IgG	IgM	IgG	IgM
1	None	120	70	360	460	2,400	2,020
	LDA_1	130	60	130	100	410	140
	MB 40.2	110	120	420	510	2,570	2,100
	L243	120	90	120	100	560	180
2	None	160	40	700	2,240	1,050	1,940
	LDA_1	200	20	50	100	300	110
	MB 40.2	130	140	650	2,300	1,160	2,010
	L243	170	30	110	140	250	100
3	None	180	160	1,100	2,800	2,600	3,460
	LDA_1	150	110	380	630	850	720
	MB 40.2	110	120	1,050	2,700	2,580	3,500
	L243	140	150	250	470	760	680

PBM (1×10^5 per reaction) from three individuals recently sensitized to TT were cultured in a round-bottomed microculture plate at 37 °C in a humidified 5% CO_2 atmosphere in RPMI 1640 medium supplemented with 15% heat-inactivated fetal calf serum (FCS), 200 mM L-glutamine, 25 mM HEPES buffer and 1% penicillin/streptomycin. PWM (Gibco) was added to the cultures to a final concentration of $10 \mu\text{g ml}^{-1}$. TT (Massachusetts Public Health Biological Laboratories) was used at a final dilution of 1:400. Cultures were tested in parallel without antibody, with anti- LDA_1 , with MB 40.2, which is an IgG1 antibody reacting with the responders HLA-B antigens, or with L243, which is of IgG2A subclass and reacts with human Ia. The amount of human IgG and IgM contained in 50- μl aliquots of day-6 culture supernatant was quantitated in round-bottomed polyvinyl trays (Costar) coated with rabbit anti-human IgG or IgM (Dako Accurate Chemicals). Known amounts of human IgG or IgM in the same medium were tested as standards. Triplicate wells were used for each reaction. Following 2 h of incubation, wells were emptied, washed in 1% FCS in phosphate-buffered saline (PBS) and covered with 50 μl of a 1:3,000 or 1:1,000 dilution of peroxidase-conjugated rabbit anti-human IgG or IgM respectively. Trays were incubated for 1 h at room temperature in the dark, then washed five times in 1% FCS/PBS, 2,2-Azino-di-(3-ethylbenzthiazoline) sulphonic acid (50 μl), diluted 1:100 in 0.1 M citrate buffer (pH 4.2) containing 0.03% hydrogen peroxidase, was added to each well. After 30 min of incubation at room temperature in the dark, 50 μl of a 1% solution of SDS was added to stop the reaction. The absorbance at 405 nm was read with an automated photometer (Flow Multiskan).

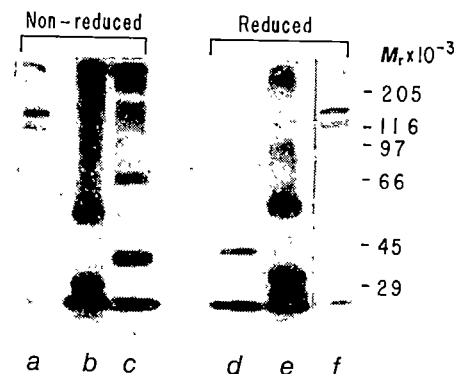


Fig. 3 SDS-polyacrylamide gel electrophoresis (PAGE) of ^{125}I -labelled membrane proteins of TCC 19 cells. 2×10^7 cells were surface labelled with 1 mCi of Na^{125}I (Amersham) using the lactoperoxidase technique as previously described¹⁴. The labelled cells were washed and lysed in 0.5 ml of 100 mM Tris-HCl, pH 7.4/1 mM phenylmethylsulphonyl fluoride/1 mM EDTA/0.5% Triton X-100. The cell lysate was centrifuged at 40,000g for 30 min and the supernatant was filtered through a 0.2- μm Millipore filter. The filtered supernatant was precleared three times by sequential 1-h incubation at 4 °C with protein A-Sepharose, rabbit anti-mouse IgG-coupled Sepharose and an irrelevant monoclonal antibody coupled to Sepharose. The precleared cell lysate supernatant was incubated for 4 h with specific antibody coupled to Sepharose-4B, following the immunoprecipitation procedure described before¹⁴. Immune precipitates were then processed for SDS-PAGE in the presence (reducing condition) of 5% 2-mercaptoethanol using a 7.5% polyacrylamide gel. Lanes a, f, anti- LDA_1 ; lanes b, e, antibody A3 to a framework determinant of human MHC class II antigen; c, d, antibody NAM 1 to human β_2 -microglobulin.

T cells recognizing class II MHC antigens comprise the population of lymphocytes which produce helper factors required for the differentiation of activated B cells into antibody-producing cells. To determine whether T-helper function is also affected by anti- LDA_1 , we tested its effect on immunoglobulin production in cultures in which peripheral blood mononuclear cells (PMB) had been primed to TT or pokeweed mitogen (PWM). Following PWM stimulation, $30 \pm 5\%$ of PWM T blasts expressed LDA_1 by day 4 and $60 \pm 8\%$ were positive by day 6. The equivalent numbers for TT blasts were 19 ± 6 and 42 ± 7 , respectively. Anti- LDA_1 had a strong inhibitory effect on immunoglobulin production in PBM cultures stimulated with PWM or TT (Table 1). Amounts of human IgG and IgM present in the day-6 supernatant of such cultures were significantly lower than those found in cultures grown without monoclonal antibodies or with monoclonal antibody MB 40.2 (ref. 19), which reacts with HLA-B antigens and was used as an isotype-matched control.

The degree of inhibition produced by anti- LDA_1 was of the same order of magnitude as that produced by monoclonal antibody L243 (ref. 20), which reacts with Ia antigens on B cells, monocytes and activated human T cells. Similarly, anti- LDA_1 was as efficient as L243 in inhibiting the capacity of helper T-cell clones possessing anti-HLA-DR¹⁵ or anti-PPD reactivity²¹ to induce B lymphocytes to produce antibodies (Table 2), but anti- LDA_1 had no effect on antibody production by activated, Epstein-Barr virus-transformed β -lymphoblastoid cell lines. Thus, for example, levels of human IgM produced by 1×10^3 B lymphoblasts from one such line in 24, 48, 72 and 96 h were 62, 114, 226 and 350 ng ml⁻¹ in cultures without antibody and 60, 140, 220 and 380 ng ml⁻¹ in cultures containing anti- LDA_1 . Immunoprecipitation studies of the molecule recognized by anti- LDA_1 were performed using ^{125}I -labelled extracts from the immunizing TCC 19. Two bands of $\sim 116,000$ and $150,000$ relative molecular mass (M_r) were found in reducing and nonreducing conditions (Fig. 3).

Our data indicate that anti- LDA_1 detects a late-differentiation antigen expressed by activated T lymphoblasts and inhibits their

Table 2 Effect of anti-LDA₁ on antibody induction by helper T-cell clones

	Immunoglobulin (ng ml ⁻¹) in cultures containing:					
	No antibody		Anti-LDA ₁		L243	
	IgG	IgM	IgG	IgM	IgG	IgM
Anti-HLA-DR1	164	135	30	19	25	18
Anti-PPD	184	98	42	26	38	15

Alloreactive T-cell clones were tested for helper function by incubating 1×10^4 cloned cells with 5×10^4 stimulator type B cells in RPMI 1640 medium containing FCS, glutamine antibodies and PWM, as described previously¹⁵. PPD-specific T-cell clones (1×10^4 cells per reaction) were incubated with autologous T-lymphocyte-depleted PBM in medium containing PPD. Triplicate cultures with no antibody, with anti-LDA₁ or with L243 were tested in parallel. Supernatants were collected from day-6 cultures and the amount of human IgG and IgM was quantitated by enzyme-linked immunosorbent assay. The amount of IgG and IgM produced by B cells alone was less than 25 ng ml⁻¹ and was subtracted from all values.

capacity to produce or deliver the helper signal(s) required for the differentiation of antibody-producing B cells. The inhibitory effect of this antibody is as strong as that displayed by anti-Ia antibodies which interfere with T-B interaction. LDA₁ may represent a cell-surface protein encoded by a 'helper' function gene or by a T-cell receptor gene (possibly Fc) involved in regulating antibody production.

Isolation of the gene and determination of the relevance of its product in autoimmune conditions, allograft rejection or T-cell malignancies is of obvious interest for the future.

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Reconstitution of functional receptor for human interleukin-2 in mouse cells

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Interleukin-2 (IL-2) has a key role in the antigen-specific clonal growth of T lymphocytes, by virtue of its interaction with a specific cell-surface receptor (IL-2R)¹⁻⁴. The growth signal seems to be delivered by IL-2 bound to the high-affinity, but not the low-affinity, receptor^{5,6}. Genes encoding IL-2 (refs 7-13) and its receptor (that is, Tac-antigen)¹⁴⁻¹⁷ have been cloned and analysed in detail. We have now achieved cell-type-specific reconstitution of the high-affinity human IL-2R by expressing the complementary DNA

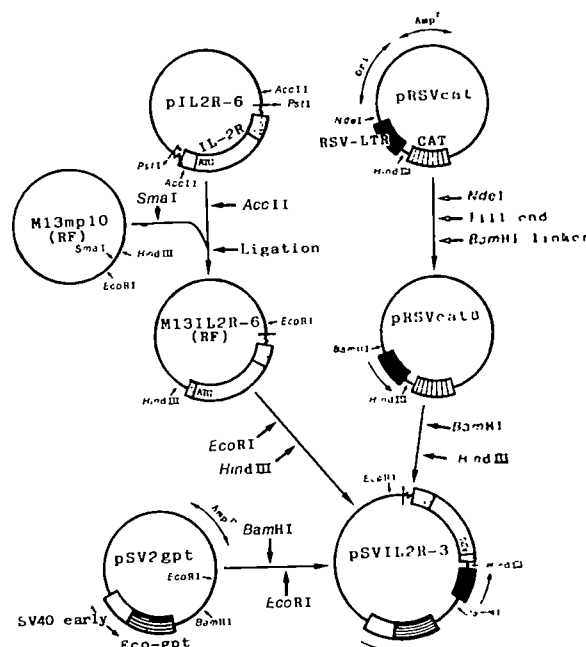


Fig. 1 Construction of the expression plasmid for human IL-2 receptor cDNA. A cDNA library complementary to poly(A)⁺ mRNA of activated human lymphocytes was constructed essentially as described previously¹⁸. Based on the published cDNA sequences for the human IL-2R¹⁴⁻¹⁶, oligonucleotide probes were synthesized and these were used to screen the cDNA library. A clone, pIL2R-6, which contains the entire coding sequence for human IL-2R, was obtained. pIL2R-6 was digested with AccII and a 1.55-kilobase (kb) IL-2R cDNA fragment was isolated. This fragment was then ligated to the SmaI site of M13 phage mp10 (M13IL2R-6). For the preparation of RSV LTR, pRSVcat²¹ was cleaved with NdeI and, after filling-in both ends using DNA polymerase I, a synthetic BamHI linker was introduced (pRSVcatB). Then the 1.6-kb EcoRI-HindIII IL-2R cDNA fragment derived from M13IL2R-6, the 0.6-kb BamHI-HindIII RSV LTR fragment derived from pRSVcatB and the EcoRI-BamHI-cleaved pSV2gpt were ligated by T₄ DNA ligase to construct pSVIL2R-3.

cloned from normal lymphocytes. A mouse T-lymphocytic line, EL-4, expressed human IL-2R with high (dissociation constant (K_d) = 160-220 pM) and low (K_d = 2.1-2.2 nM) affinity for recombinant human IL-2, while mouse L929 cells expressed only a single class of the IL-2R with lower affinity (K_d = 34.5 nM) for the ligand. We also show that the human IL-2R expressed in EL-4 cells responds to IL-2 and mediates reversed signal transduction: growth of the EL-4 cells harbouring the IL-2R is inhibited specifically by human recombinant IL-2. The approach described here may provide a general experimental framework for elucidating the molecular basis of signal transduction mediated by specific receptor-ligand interaction.

We prepared a cDNA library using poly(A)⁺ RNA from activated human lymphocytes¹⁸ and screened it for IL-2R (Tac-antigen)-specific clones with oligonucleotide probes. Among several positive clones identified, clone pIL2R-6 was analysed and shown to contain a nucleotide sequence encoding a complete polypeptide identical to that of aberrantly expressed IL-2R in leukaemic cells derived from adult T-cell leukaemia (ATL), reported previously by others¹⁴⁻¹⁶ (data not shown).

Recent studies using radiolabelled IL-2 demonstrated the existence of two classes of IL-2R that differ in their affinities for IL-2 (refs 5, 6). The finding that IL-2-induced T-cell proliferation correlates well with the expression of the high-affinity receptor suggests that the high-affinity receptor alone is capable of signal transduction^{1,2,5}. Although it was reported previously that the cloned human IL-2R cDNA can direct the expression of a cell-surface molecule capable of binding both IL-2 and a monoclonal anti-human IL-2R antibody, anti-Tac^{19,20}, in cultured monkey kidney (COS) cells^{14,15}, it was unclear whether that molecule was functioning as IL-2R, that is, whether it

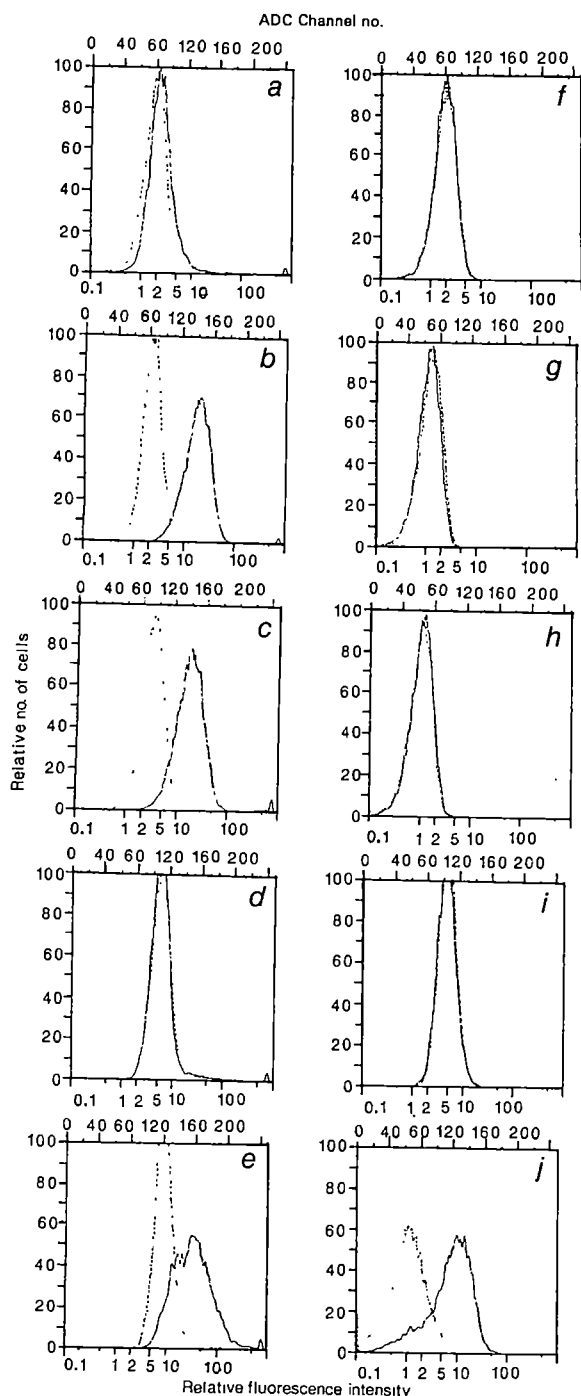


Fig. 2 Flow microfluorimetric analysis of mouse cells expressing either human or mouse IL-2 receptor. Mouse cells were stained indirectly with either mouse anti-human IL-2R monoclonal antibody, anti-Tac (*a-e*), or rat anti-mouse IL-2R monoclonal antibody, AMT-13 (*f-j*). Cells used for staining were as follows: *a, f*, EL-4; *b, g*, ELT-5 (an EL-4 transformant); *c, h*, ELT-13 (an EL-4 transformant); *d*, L929; *e, i*, LCT-4 (an L929 transformant); *j*, concanavalin A-activated splenocytes (Con A blasts) of CBN mouse origin. Dotted lines represent staining with the fluorescent second-step conjugate alone.

Methods. Cells (5×10^5) were incubated in 50 μ l Hank's balanced salt solution containing 0.1% bovine serum albumin and 0.1% NaN_3 (HBSS-BN) for 30 min at 4°C with saturating concentrations of anti-Tac (1:500 dilution of ascites fluid) or AMT-13 (1:500 dilution of ascites fluid). After incubation, cells were washed three times, then resuspended in 40 μ l HBSS-BN containing appropriate concentrations of fluorescein-conjugated goat anti-mouse IgG or mouse anti-rat κ -chain for 30 min at 4°C. Then the cells were washed three times, resuspended in 0.5 ml HBSS-BN and analysed using a FACS 440 (Becton Dickinson). Con A blasts were prepared as described previously²⁵. EL-4 transformants were produced by transfecting pSVIL2R-3 into EL-4 by the protoplast fusion technique essentially as described by Oi *et al.*³². Mycophenolic acid ($4 \mu\text{g ml}^{-1}$)-resistant colonies were visible by 10–14 days; these cells were cloned by the limiting dilution method. Transfection of pSVIL2R-3 into L929 cells was done using the calcium phosphate technique as described by Chu and Sharp³³. After 3 weeks, mycophenolic acid ($2.5 \mu\text{g ml}^{-1}$)-resistant cells were collected, stained indirectly with anti-Tac antibody and the positive cells were cloned by single-cell manipulation using FACS, as described by Parks *et al.*³⁴.

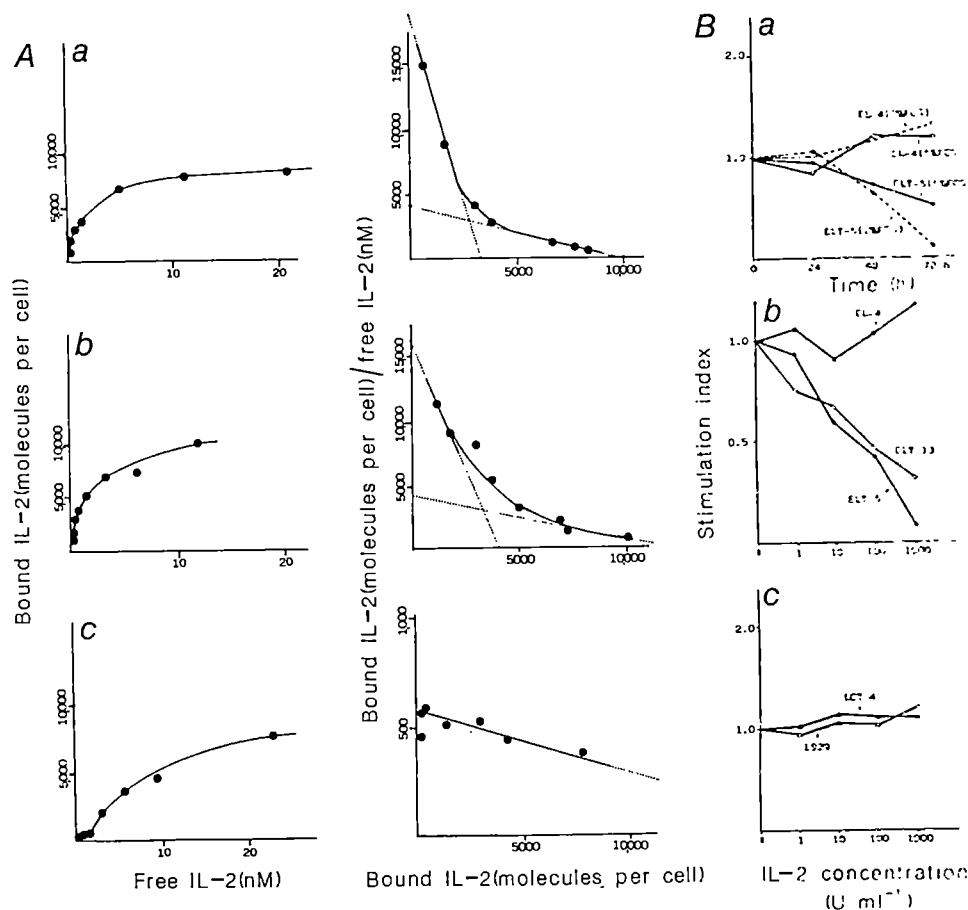
exhibited high affinity for IL-2 and delivered the IL-2-specific signal.

To express the IL-2R cDNA stably in mammalian cells, we first constructed a plasmid, pSVIL2R-3, as shown in Fig. 1. Into this plasmid we inserted the long terminal repeat (LTR) sequence of Rous sarcoma virus (RSV)²¹ just upstream of the IL-2R cDNA and introduced this chimaeric gene into the pSV2gpt vector²². This plasmid was transfected into EL-4 cells, a benzo[*a*]pyrene-induced thymoma line²³, and into mouse L929 cells, a transformed fibroblast cell line. Transformants were selected in medium containing mycophenolic acid (MPA) and cells expressing the human IL-2R (Tac antigen) were cloned. Blotting analysis of the DNA prepared from each of 16 EL-4 and 10 L-cell transformant clones indicated the presence of one to two copies of the transfected human cDNA (data not shown). Three of these transformed cell clones, ELT-5, ELT-13 (EL-4 origin) and LCT-4 (L929 origin), were arbitrarily chosen for further analysis. As shown in Fig. 2*a-e*, immunofluorescent staining profiles clearly demonstrate the expression of Tac anti-

gen on the cell surface of IL-2R cDNA transformants, while parental cells are negative for the same antigen.

To determine whether the human IL-2R expressed on the surface of murine cell transformants manifests high or low affinity for IL-2, we performed radiolabelled IL-2 binding assays. Scatchard plot analysis of two EL-4 transformants showed curvilinear features, demonstrating the presence of two distinct receptors having different binding affinities for recombinant IL-2, with dissociation constants of 160 and 2,100 pM in ELT-5, and 220 and 2,200 pM in ELT-13 (Fig. 3*A, a, b*). This result was highly reproducible and, in our assay system, the K_d of the high-affinity receptor expressed on activated human T lymphocytes and some ATL cells falls within the range 10–100 pM⁶. Thus, the K_d of the high-affinity receptor expressed on the heterologous mouse cell transformants is only slightly lower than that of the endogenous high-affinity receptor on human cells, and the K_d of the second class of receptors expressed on transformant cells is also similar to that of the low-affinity receptor expressed on human cells⁶. In contrast to the EL-4

Fig. 3 **A**, IL-2 binding assay. EL-4 and L929 cell transformants expressing human IL-2R were examined for their IL-2 binding activities using radiolabelled human IL-2. ELT-5 (**a**) and ELT-13 (**b**) were derived from EL-4, and LCT-4 (**c**) was derived from L929. The binding curve of radiolabelled IL-2 (left) and the Scatchard plot analysis of the IL-2 binding data (right) are shown. Human recombinant IL-2 (purity >99.8%) was radiolabelled using ^{125}I -Bolton and Hunter's reagent. The specific radioactivity of the labelled IL-2 was 2,000–5,000 c.p.m. ng^{-1} . The ^{125}I -IL-2 binding assay was performed using methods described by Robb *et al.*⁵, with slight modifications⁶. Serial dilutions of radiolabelled IL-2 and 2×10^6 cells were incubated for 40 min at 37 °C in a total volume of 0.5 ml of RPMI 1640 medium containing 25 mM HEPES, 1% bovine serum albumin (BSA) and 0.1% sodium azide. After the incubation, cells were washed and resuspended in the same medium. The cell suspension (200 μl) in duplicate was layered over 150 μl of a mixture of 20% olive oil and 80% di-n-butylphthalate. Following centrifugation of samples, the tips of the sample tubes containing the cell pellets were cut off and the radioactivities were measured using a γ -counter. Nonspecific binding was determined by incubating cells in the presence of a 300-fold excess of unlabelled IL-2. Specific binding was obtained by subtracting nonspecific binding, which represents ~10% of the total binding. **B**, Effect of human recombinant IL-2 on the mouse cells expressing human IL-2R. **a**, Time course kinetics of the effect of IL-2 (1,000 U ml^{-1}) on the growth of EL-4 (\circ) and ELT-5 (\bullet) in medium supplemented with 2% (—) and 5% (---) fetal calf serum (FCS). **b**, **c**, Dose-response curves of IL-2. Cells were cultured for 72 h in medium containing 10% FCS in the presence of various concentrations of IL-2. Cells used were EL-4 (\blacksquare), ELT-5 (\bullet), ELT-13 (\circ), L929 (Δ) and LCT-4 (\blacktriangle). EL-4 and its transformants, ELT-5 and ELT-13, were grown in RPMI 1640 medium supplemented with 10% FCS. L929 and the transformant LCT-4 were cultured in ES medium (Nissui) containing 10% FCS. Cells collected from the culture were plated at 1×10^3 per well in 96-well flat-bottomed microculture plates. Cells were cultured in RPMI 1640 or ES medium in the presence of various concentrations of human recombinant IL-2. Half of the medium was aspirated daily and was replaced with fresh medium containing the same supplements; 4 h (EL-4 and its transformants) or 8 h (L929 and LCT-4) before the termination of culture, 1 μCi of ^3H -thymidine (^3H -TdR) was added to each culture well. Then cells were placed on glass fibre strips using an automatic cell harvester and ^3H -TdR incorporation was measured as an index of cell proliferation. Results are expressed as a stimulation index (SI) = c.p.m. ^3H -TdR incorporation with IL-2/c.p.m. ^3H -TdR incorporation without IL-2. The specific activity of the human recombinant IL-2 was 5×10^7 U per mg protein.



transformants, a mouse L929 transformant, LCT-4, expressed only a low-affinity receptor having a K_d of 34.5 nM (Fig. 3A, c); interestingly, this value is one order of magnitude higher than that of the low-affinity receptor expressed on the EL-4 transformants. The total number of IL-2R molecules per cell was approximately 9,000, 12,000 and 20,000 for ELT-5, ELT-13 and LCT-4, respectively (Fig. 3A). As regards the high-affinity receptor, we found 3,000 molecules per cell in ELT-5 and 4,500 molecules per cell in ELT-13. Thus, the number of IL-2R molecules expressed in these transformants is similar to that of activated human T cells and one order of magnitude lower than that of leukaemic T cells such as MT-1 or HUT-102 (refs 5, 6). As mouse IL-2R is known to react with human IL-2 (ref. 24), we determined whether EL-4 cells and the transformants express mouse IL-2R on their surface. A binding assay using ^{125}I -labelled human recombinant IL-2 demonstrated no significant binding of IL-2 with parental EL-4 cells (data not shown). Moreover, fluorescence-activated cell sorter (FACS) analysis using rat anti-mouse IL-2R monoclonal antibody, AMT-13 (ref. 25), revealed no mouse IL-2R (Fig. 2f–j). These results demonstrate that high-affinity human IL-2R can be reconstituted in heterologous host cells by expression of the cDNA encoding the Tac antigen and that expression of the high-affinity IL-2R requires a T-cell-specific background.

The fact that introduction of human IL-2R cDNA into EL-4

cells leads to constitutive expression of the high-affinity receptor for human IL-2 prompted us to examine whether the human IL-2/receptor system delivers the signal transduction in mouse cells. When recombinant human IL-2 was added to culture media of the transformants, it inhibited the growth of ELT-5 and ELT-13 cells, as expressed by incorporation of ^3H -thymidine into DNA, but had no significant effect on parental EL-4 cells (Fig. 3B, a, b). The growth inhibition of EL-4 transformants was evident at an IL-2 concentration of 1–10 U ml^{-1} (~1.3–13.0 pM), at which concentration IL-2 should bind only to the high-affinity receptor (Fig. 3B, b). Thus, it is likely that the signal transduction is mediated through the high-affinity receptor. At a higher concentration of IL-2 (1,000 U ml^{-1} ; 1.3 nM), a reduction of almost 90% in ^3H -thymidine incorporation was observed. At this IL-2 concentration, growth of the EL-4 transformants was detectably inhibited by 48 h after the onset of culture with IL-2 and ceased almost completely by 72 h (Fig. 3B, a). Cell viability at that time was over 95% and cell proliferation was resumed after removal of IL-2 from the culture media (data not shown). This growth inhibition was not due to growth inhibitory substances whose production is induced by IL-2, as growth of parental EL-4 cells was not inhibited by co-cultivation with ELT-5 or ELT-13 in the presence of 1,000 U ml^{-1} IL-2 (data not shown). In contrast to the observations with these EL-4 transformants, no significant growth alteration was observed for either LCT-4 cells or parental

Table 1 Effect of anti-Tac antibody on the IL-2-induced growth inhibition of ELT-5 cells

Cell line	Treatment		c.p.m. (mean \pm s.d.)	SI
	IL-2	Anti-Tac		
EL-4	-	-	27,600 \pm 1,300	1.00
	500 U ml ⁻¹	-	29,900 \pm 2,200	1.08
	-	1 μ g ml ⁻¹	27,700 \pm 2,400	1.00
	500 U ml ⁻¹	1 μ g ml ⁻¹	29,500 \pm 1,900	1.07
ELT-5	-	-	29,900 \pm 1,300	1.00
	500 U ml ⁻¹	-	5,100 \pm 500	0.17
	-	1 μ g ml ⁻¹	29,700 \pm 4,300	0.99
	500 U ml ⁻¹	1 μ g ml ⁻¹	27,800 \pm 1,300	0.93

The cell proliferation assay was performed as described in the legend to Fig. 3B. Anti-Tac antibody was added at the onset of culture. After 24 and 48 h, half of the medium was removed from each culture and replaced with fresh medium containing the same supplements (IL-2 and/or anti-Tac). After 72 h, 1 μ Ci of ³H-thymidine was added to each culture well, incubation was continued for a further 4 h and the ³H-thymidine incorporation was measured. The stimulation index (SI) was determined as follows; SI = ³H-thymidine incorporation with treatment/³H-thymidine incorporation without treatment.

L929 cells (Fig. 3B, c). As stated above, EL-4 and its transformants do not detectably express mouse IL-2R on their cell surface, therefore growth inhibition of the EL-4 transformants induced by human recombinant IL-2 is most likely mediated by the human IL-2R. This conclusion is reinforced by the finding that the addition of anti-Tac antibody to culture medium containing IL-2 restores almost completely the growth of ELT-5 cells (Table 1). As expected, AMT-13, which recognizes the mouse IL-2R and blocks IL-2-dependent murine T-cell growth²⁵, had no effect on the human IL-2-mediated growth inhibition of ELT-5 (data not shown).

Bifunctional properties of growth factors have been reported also for other growth factor/receptor systems such as that of epidermal growth factor (EGF)^{26,27} and transforming growth factor type β ²⁸. Growth of A431 human epidermal carcinoma cells, which express an unusually high number of EGF receptors, is inhibited in the presence of a high concentration of EGF²⁶. The mechanism of growth inhibition in that system may be related to overstimulation of receptor-associated tyrosine kinase²⁷. As the intracytoplasmic portion of the IL-2R is too short to mediate any kinase activity¹⁴⁻¹⁶, the mechanism of growth inhibition reported here is likely to be different. Recently, high concentrations of IL-2 were reported to inhibit growth of some but not all HTLV (human T-cell lymphotropic virus)-infected T-cell clones, by interacting with IL-2R²⁹. It is illuminating that the growth factor/receptor interaction in neoplastic cells, which are capable of uncontrolled cell proliferation, often leads to reversal of the growth stimulatory signal. As we used a viral LTR sequence for the IL-2R cDNA expression, a 'down-regulatory' mechanism of the heterologous receptor will not operate. Indeed, the level of IL-2R mRNA and Tac-antigen remained unchanged in the presence of IL-2 in ELT-5 cells (our unpublished observations). Thus, continuous growth factor/receptor interaction on the cell surface may also account for the inhibition of the uncontrolled cell growth. Such a reversed signal transduction may be delivered by activation of protein kinase C via a high-affinity receptor³⁰.

The expression of functional, specific receptor molecules introduces a powerful new tool for the investigation of ligand-receptor interactions, and especially for extending our understanding of molecular mechanisms of signal transduction by means of site-directed mutagenesis of the cloned genes and functional study of the gene product.

After submission of this manuscript, Greene *et al.* reported the expression of low-affinity IL-2Rs in mouse L-cells³¹.

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Human blood platelets showing no response to collagen fail to express surface glycoprotein Ia

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The interaction of blood platelets with collagen is generally considered to be of primary importance in the arrest of bleeding and to have a role in the pathogenesis of thrombosis and atherosclerosis. Following damage to the vascular endothelium, circulating platelets come into contact with exposed collagen fibrils in the sub-endothelium and spread along it; this is followed by the secretion of several biologically active substances and by aggregation of platelets. The glycoproteins of the platelet plasma membrane have an important role in the mechanisms underlying these processes. So far, two specific defects of platelet function in patients with a bleeding disorder are known to be associated with a glycoprotein defect and the study of these patients has contributed significantly to present concepts of platelet function. The glycoprotein (GP) IIb-III complex, absent or deleted in the aggregation-defective Glanzmann's thrombasthenia¹, has been identified as the platelet fibrinogen receptor. GPIb, which is absent in the adhesion-defective Bernard-Soulier syndrome^{2,3}, has been identified as the von Willebrand factor receptor on platelets. We now report a defect of the platelet plasma membrane glycoprotein composition in a patient whose platelets are totally unresponsive to collagen.

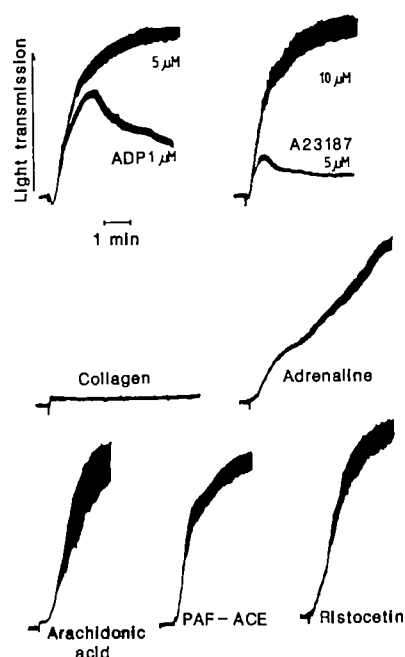


Fig. 1 Aggregation tracings of patient's platelet-rich plasma. Aggregation studies were performed at 37 °C and were normal in response to ADP (1 and 5 μ M), A23187 (5 and 10 μ M), adrenaline (5 μ M), arachidonic acid (1.5 mM), PAF-ACE (1 μ M) and ristocetin (1 mg ml⁻¹). Collagen induced neither shape change nor aggregation.

We studied the platelets of a patient with a haemorrhagic disorder and an excessively long bleeding time (>30 min, compared with the normal range of up to 8 min). Platelet count, coagulation studies and factor VIII parameters were normal. With the informed consent of the patient and control subjects, venous blood was collected in 0.1 vol. of 135 mM trisodium citrate. For some experiments the platelets were isolated by passage through a Sepharose 2B column (Pharmacia) equilibrated in Ca²⁺-free Tyrode's solution (pH 7.2). Aggregation in the patient's platelet-rich plasma (PRP) (Fig. 1) was normal in response to ADP, the ionophore A23187, adrenaline, arachidonic acid, PAF-ACE (platelet aggregating factor/alcohol-chloroform-ether mixture) and ristocetin. Thrombin-induced aggregation was normal in gel-filtered platelet suspensions. In contrast collagen induced neither shape change nor aggregation in PRP and gel-filtered platelet suspensions. This result was found consistently for both low and very high concentrations of several types of fibrillar collagen: equine collagen type I (1–10 μ g ml⁻¹), bovine collagen types I (5–100 μ g ml⁻¹) and III (50–100 μ g ml⁻¹) as well as with purified human collagen types I and III (10–100 μ g ml⁻¹). Normal platelets aggregated with the lowest concentrations of these collagen types. Experiments in which PRP from the patient was mixed with control platelet-free plasma or in which platelet-free plasma from the patient was added to control PRP, demonstrated the absence of a circulating inhibitor and suggested that the defect resided in the platelets.

As current data suggest that the breakdown of phosphatidylinositol (PI) with concurrent formation of diacylglycerol and phosphatidic acid (PA) might be an early common mechanism whereby activation of receptors brings about physiological responses, we studied the metabolism of PI in the patient's platelets (Table 1). In normal platelets labelled with ³²P-orthophosphate, stimulation caused an increase in the ³²P content of PA and PI, which suggests that the PA formed recycles to PI. Treatment of normal ³H-arachidonate-labelled platelets with thrombin or collagen caused loss of radioactivity from PI, with a corresponding accumulation of radioactive PA, free arachidonate and its oxygenation products. In the patient's platelets, thrombin (5 U ml⁻¹) induced a normal decrease of ³H-PI, a normal accumulation of ³²P-PA, a normal incorporation

Table 1 Changes in distribution of label in platelets prelabelled with ³²P-orthophosphate and ³H-arachidonate after stimulation (5 min, 37 °C) with thrombin (5 U ml⁻¹) or collagen (10 μ g ml⁻¹)

	Controls	Patient	
Thrombin			
³ H-PI	33 ± 7%	44%	NS
³² P-PI	146 ± 21%	155%	NS
³² P-PA			
(before exposure)	3,200 c.p.m. per 10 ⁹ pl.	2,100 c.p.m. per 10 ⁹ pl.	
(after exposure)	76,100 c.p.m. per 10 ⁹ pl.	44,300 c.p.m. per 10 ⁹ pl.	
Collagen			
³ H-PI	59 ± 9%	98%	<i>P</i> < 0.05*
³² P-PI	133 ± 15%	95%	<i>P</i> < 0.05*
³² P-PA			
(before exposure)	3,200 c.p.m. per 10 ⁹ pl.	2,100 c.p.m. per 10 ⁹ pl.	
(after exposure)	32,400 c.p.m. per 10 ⁹ pl.	2,000 c.p.m. per 10 ⁹ pl.	

Data are expressed as a percentage (mean ± s.d.) of the radioactivity before activation for ³H-PI and ³²P-PI (for controls, *n* = 5) and, because of low basal values, are given as c.p.m. per 10⁹ platelets (pl.) for ³²P-PA. The ³²P-PA control data are representative of 10 controls studied. Platelets were incubated with 0.01 μ M ³H-arachidonate (specific activity 90 Ci mmol⁻¹; TRK, Amersham International) and 0.1 mCi (carrier-free) H₃³²PO₄ (NEN) for 60 min at 37 °C. Twenty per cent of the radiolabelled arachidonate was taken up by the platelets. After gel filtration the labelled platelets were incubated with thrombin or collagen for 5 min. Extracts were obtained and the phospholipids, fatty acids and thromboxanes were separated by TLC as described elsewhere¹⁹. The ³H and ³²P content of the fractions was determined by scintillation counting.

* Significant difference by Student's *t*-test; NS, not significant (*P* > 0.05).

of ³²P in PI and a normal liberation of ³H-arachidonic acid. Changes in the PI cycle and liberation of ³H-arachidonic acid were not observed after exposure of the patient's platelets to collagen. Thromboxane B₂ (TXB₂) was produced normally in the patient's PRP in response to exogenous arachidonate (1.5 mM), as determined by radioimmunoassay. After incubation for 1 min, the patient's PRP generated 540 nmol TXB₂ per 10¹¹ platelets (normal range 110–518 nmol per 10¹¹ platelets, *n* = 8). There was no detectable TXB₂ generation on stimulation of the patient's platelets with collagen for 5 min (patient < 2, normal range 90–142 nmol per 10¹¹ platelets, *n* = 8).

The interaction of blood platelets with purified human fibrillar collagen type I in a static system was studied according to Legrand⁴ using a technique based on gel filtration of non-adhesive platelets through a Sepharose 2B column after incubation of PRP with fibrillar collagen. For the slightly modified test used here, 0.8 ml of PRP (4 × 10⁵ platelets μ l⁻¹), obtained from EDTA-blood to prevent aggregation, was incubated (37 °C, 2.5 min, without stirring) with 0.2 ml of 0.02 M sodium phosphate buffer pH 7.4 with and without 100 μ g of purified human collagen type I, made fibrillar as described elsewhere⁵. The mixtures were applied to a Sepharose 2B column, equilibrated in a Ca²⁺-free Tyrode's solution, pH 7.4, containing 20 mM HEPES and 0.2% (w/v) bovine serum albumin. The platelets were counted before and after passage and the percentage adhesion calculated. After incubation of the patient's platelets with collagen and subsequent gel filtration, almost no platelets were retained with the collagen fibrils at the top of the column: there was 4.4 ± 0.7% adhesion in the patient, 83.4 ± 1.0% in the control (mean ± s.d. of triplicate experiments). This severe defect in adhesion is present in a static system, where platelet transport towards collagen occurs as a result of diffusion and gravitation, and consequently it differs from the shear-dependent adhesion defects in von Willebrand's disease and Bernard-Soulier syndrome.

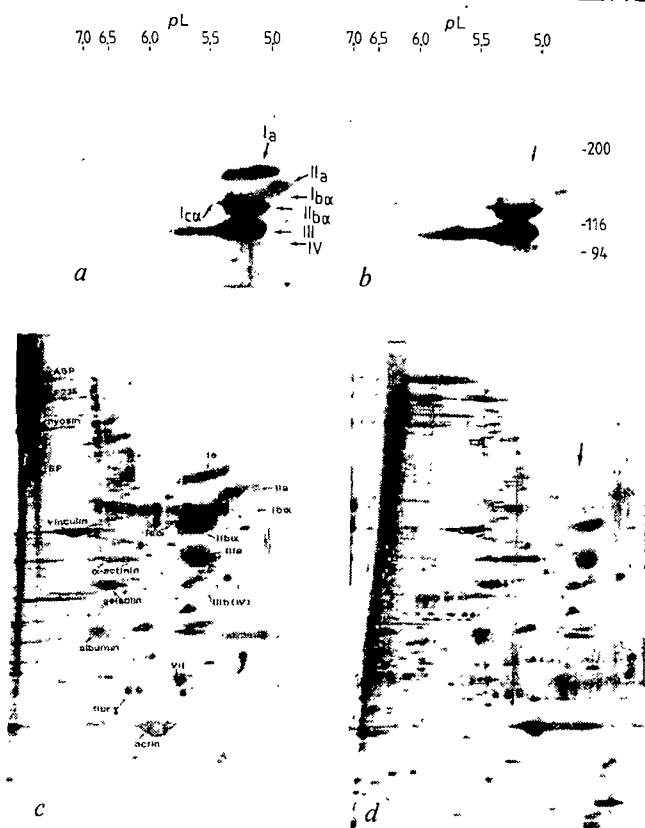


Fig. 2 Autoradiograms (*a, b*) and silver staining (*c, d*) of two-dimensional reduced SDS-polyacrylamide gels for control (*a, c*) and patient (*b, d*) platelets. The positions of the major membrane glycoproteins are indicated. Note that GPIa is lacking in the patient. Values shown on the right-hand side in *b* are $M_r \times 10^{-3}$. **Methods.** Platelets were radioiodinated in parallel by using Iodogen (Pierce Chemicals, Rockford, Illinois) as a catalyst. Isoelectric focusing, SDS-polyacrylamide gel electrophoresis (3–30% acrylamide linear gradient gels) and autoradiography of the gels were performed as described elsewhere²⁰. Gels were silver-stained using a technique described previously²¹.

Analysis of the platelet ectoproteins by silver staining and ¹²⁵I surface labelling, coupled with one- and two-dimensional gel electrophoresis, disclosed a deficiency of the membrane glycoprotein Ia (relative molecular mass (M_r) 168,000 under reducing conditions, pI 4.8–5.4) (Fig. 2). Similar results were obtained when repeated labellings of the patient's platelets were compared with the autoradiograms obtained from 10 controls. Extreme overexposure of the autoradiograms revealed a small amount of residual radioactivity in the GPIa region. To quantitate the amount of label in GPIa, this glycoprotein was marked in the gels by autoradiography, cut out of the two-dimensional gels, and counted in a scintillation counter. Several other glycoproteins in the gels were used as standards. Consistent results were obtained on radiolabelling of normal platelets (GPIIb/GPIIIb = 0.35 ± 0.04 (\pm s.d.); GPIa/GPIIIb = 0.22 ± 0.06 , $n = 5$). Repeated simultaneous labelling studies of the patient's platelets revealed 15–25% of the normal level of GPIa. Low but detectable amounts of GPIb (7–30%) and GPIIb–III (12–25%) have been found in some patients with Bernard-Soulier syndrome^{3,6,7} and Glanzmann's thrombasthenia^{8,9}. These patients, referred to as type 2, suffer from a bleeding syndrome and have abnormal (absent) aggregation responses. The defect in the patient studied here—a complete lack of response to collagen associated with a markedly reduced concentration of GPIa—resembles these corresponding platelet function defects in this respect.

GPIa is an ectoprotein which contains intramolecular disulphide bonds as it has an increased apparent relative molecular mass after disulphide cleavage. GPIa is weakly stained by the periodic acid-Schiff reaction, weakly radiolabelled by the perio-

date method and is hardly affected by treatment with neuraminidase, which indicates that it contains a relatively low concentration of sialic acid. GPIa binds to the lectin wheat-germ agglutinin (*N*-acetylglucosamine specificity)¹⁰.

A putative role as the platelet receptor for collagen has been proposed for glucosyltransferase¹¹, fibronectin¹², complement component C1s¹³, a protein of M_r 65,000 (ref. 14), and glycoprotein IIb¹⁵. Subsequent studies, however, do not confirm such a role for glucosyltransferase^{16,17} or for fibronectin¹⁸ and it is unlikely that GPIIb serves as a collagen receptor, as in Glanzmann's thrombasthenia (a disorder with a deficiency of the fibrinogen receptor, GPIIb–GPIIIb), collagen-induced shape change and release are normal. In the case of the patient studied here it is evident that the primary interaction of the platelet with collagen is disturbed. In contrast to collagen, several other agonists induced normal physiological responses in the patient's platelets. These results indicate that after exposure to collagen, information could not be transmitted across the plasma membrane to trigger the intracellular events. The observed membrane abnormality occurs in parallel with the lack of responsiveness to collagen, which suggests a cause and effect relationship. Consequently, GPIa may have a receptor or transmitter function.

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Specific growth response of *ras*-transformed embryo fibroblasts to tumour promoters

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Chemical carcinogenesis is a process involving multiple steps, as shown in several *in vivo* experimental systems¹. Two early steps have been well characterized: initiation, achieved by a single, subthreshold dose of a carcinogen, and promotion, induced by repetitive treatments with a non-carcinogenic tumour promoter. At the cellular level, establishment of the transformed phenotype is also a multi-step process and activation of several, independent genes appears to be required^{2–4}. Here we show that, like initiated cells, primary rat embryo fibroblasts (REFs) containing a *ras* but

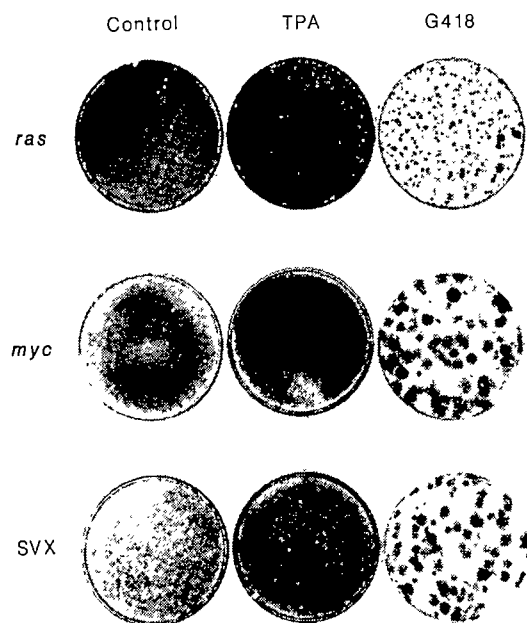


Fig. 1 Effect of TPA on focus formation of oncogene-bearing REFs in monolayer. Tertiary rat embryo fibroblasts (7×10^5 cells per 10-cm dish) were infected with the SVX, raszip 6 or VM viruses (MOI = 0.1–1). After 48 h, cells were split 1:10 into normal medium (Dulbecco's modified essential medium/10% fetal bovine serum), medium plus TPA (10 ng ml^{-1}), or medium plus G418 (0.5 mg ml^{-1}). Dishes were fixed and stained 10 days later.

not a *myc* oncogene, are strongly and specifically stimulated to grow by tumour promoters. In the presence of these promoters, *ras*-containing REFs acquire the ability to overgrow normal cells in the monolayer and to form foci with 100% efficiency. Similar to the *in vivo* situation, promoter effects can be blocked by the concomitant application of retinoic acid.

One of the best *in vivo* models for chemical carcinogenesis is represented by the mouse skin system^{1,5,6}, in which a minimum of two genetic lesions seems to be involved in tumour formation⁷. An apparent parallel arises from the observation that full transformation of primary embryo fibroblasts in culture occurs after introduction of two genetic elements—a *ras*-like and a *myc*-like oncogene. Cells bearing the two oncogenes are fully tumorigenic *in vivo*.

The mouse skin model of initiation and promotion carries specific implications for the action of the promoter and its effects on initiated cells⁵. According to this model, the initiating carcinogen creates a small number of initiated cells, each one bearing a lesion in a specific gene. The promoter then acts to induce the clonal expansion of each initiated cell, which results in a large descendant population, in one cell of which a rare second genetic lesion may now occur. On this basis, an initiated cell is one which enjoys a special growth advantage in the presence of a promoter; and a promoter is a compound that selectively stimulates the outgrowth of already initiated cells.

We reasoned that activation of a *ras*-like or *myc*-like oncogene might represent the initiating event. As a model in which to test this hypothesis, we produced REFs carrying either a *ras* or a *myc* oncogene. We aimed to measure whether either of these cell types enjoyed a special growth advantage in the presence of a tumour promoter such as TPA (12-*O*-tetradecanoylphorbol-13-acetate).

Cells carrying recently introduced oncogenes are often elusive in REF cultures, as their presence is not always signalled by the outgrowth of a focus of transformants². Therefore, we additionally marked these cells with the gene which confers resistance to killing by the antibiotic G418 (ref. 8). This would allow us to quantitate the number of oncogene-bearing cells in a culture at any point by measuring the number that were resistant to the antibiotic (G418^r).

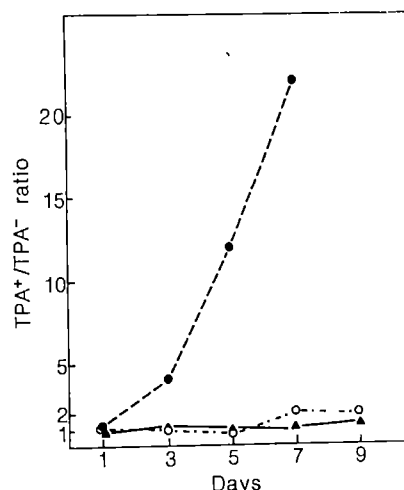


Fig. 2 Effect of TPA on the growth of oncogene-bearing REFs in monolayer. REFs were infected as described in Fig. 1 legend. After 48 h the cells were split 1:10 into either normal or TPA-containing medium and separate dishes were withdrawn at various times after that. Cells were trypsinized, counted and re-plated at a density of 1 and 5×10^5 cells per 10-cm dish in G418-containing medium (0.5 mg ml^{-1} ; Gibco). Colonies were counted after 2 weeks and the ratio of the number of colonies from cells grown with TPA to the number from cells grown without TPA was calculated. Two independent dishes were scored for each experimental point. Values are given for cells infected with raszip 6 (●), VM (○) and SVX (▲).

In previous work with REFs, DNA transfection had been used to introduce oncogenes². However, the efficiency of gene transfer by this method is low, and the number of transfected cells hard to control. Therefore, we used retrovirus vectors to convey both oncogenes and the G418^r marker into these cells. By transfecting the resulting viral genomes into $\psi 2$ cells⁹, we obtained high-titre recombinant viruses that were free of helper. These vectors could introduce oncogenes into REFs but would not allow virus spread beyond the initially infected cell.

One retrovirus carrying the *ras* oncogene was constructed by inserting the viral Ha-*ras* gene (map positions 4,000–4,710; ref. 10) into the *Bam*HI site of SVX, a retroviral vector already containing a G418-resistance gene¹¹. The DNA construct was transfected into $\psi 2$ cells⁹, resulting in a virus stock (raszip 6) showing a high titre of induction of G418 resistance (10^6 – 10^7 colony-forming units ml^{-1}). When the virus was tested on established NIH 3T3 mouse cells, acquisition of resistance to G418 was always accompanied by a morphological transformation characteristic of that induced by the *ras* oncogene. A second virus used in these studies, VM, carries the *myc* oncogene (p110 *gag-myc* fusion gene from pv-*myc*²) in addition to the G418^r marker. This virus induces expression of *myc* transcript and protein and is able to immortalize REFs in preparation). As a control, we used the SVX virus¹¹, which carries only the G418 resistance marker and no oncogene.

Third-passage REFs were infected with the three viruses at various concentrations and after 2 days were split into two groups which were then grown in either normal medium or medium containing G418 (0.5 mg ml^{-1}). After incubation for 1 week, dishes were scored either for foci or for colony formation in selective medium.

No foci could be detected in monolayers carrying the *myc* virus, independent of the multiplicity of infection (MOI). Similarly, when the *ras* virus was applied at moderate MOI, resulting in the infection of only 0.1–1% of the cells, no foci were seen. This result agrees with earlier work in which transfection was used to introduce the *ras* oncogene into a small proportion of the REF cells, yielding no observable foci².

We then assessed the effects of TPA on these various populations of oncogene-bearing and normal cells. TPA was used at

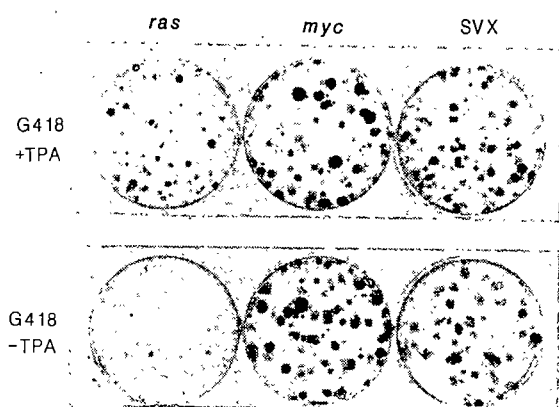


Fig. 3 Effect of TPA on the growth of oncogene-bearing REFs in the absence of surrounding normal cells. Cells were infected as described in Fig. 1 legend and after 48 h were split 1:10 into G418-containing medium (0.5 mg ml^{-1}), with or without TPA (10 ng ml^{-1}). Dishes were stained 1 week later.

10 ng ml^{-1} , a concentration comparable to that normally used to study the physiological effects of this substance on cells in culture¹².

TPA had no effects on the ability of SVX or *myc*-bearing cells to form foci in the monolayer (Fig. 1). In contrast, the effects on *ras*-bearing cells were dramatic, in that large numbers of foci of morphologically transformed cells were now apparent in the monolayer (Fig. 1). Significantly, the number of foci closely approximated the number of G418^r colonies obtained from the same cell population when plated on selective medium (Fig. 1). Most of the cells in these colonies showed a transformed morphology (data not shown). Thus, almost every cell that expressed the G418^r gene of the raszip 6 chimera was also able to form a focus in the presence of TPA. This ability of TPA to affect all the '*ras* cells' present in the monolayer conforms to epigenetic effects associated with promoters and contrasts with the rarely occurring genetic alterations associated with initiating agents¹.

The appearance of these foci suggested that the tumour promoter allowed the *ras* transformants to overcome the growth inhibitory effect imposed on them by the surrounding monolayer of normal cells. We wished to substantiate this view by direct measurement of the number of *ras* (or *myc*)-transformed cells growing in these culture conditions. Cultures infected with SVX or *ras* or *myc* virus were grown for various times in the presence or absence of TPA, then trypsinized and part of them re-plated in the presence of G418, but with no TPA in the medium. This allowed us to measure the proportion of SVX, *ras* or *myc*-bearing cells in the population, independently of any assay of focus-forming ability. It is clear from Fig. 2 that the proportion of '*myc*-cells' was not substantially affected by the presence of TPA. Similarly, the proportion of control SVX-infected cells was also not changed by TPA. In contrast, the proportion of '*ras*-cells' in the cell population was much greater in the presence of the tumour promoter; it was 4-fold higher than the control 3 days after the beginning of the experiment, 10-fold higher at 5 days and 20-fold higher at 7 days. This result demonstrates directly that the clonal expansion of cells containing a *ras*, but not a *myc* oncogene, is strongly and preferentially stimulated by the presence of tumour promoter in the medium.

These data suggest that TPA might act directly on the *ras* transformants, stimulating their growth and thereby allowing them to overcome the inhibitory effects of adjacent normal cells. Alternatively, the promoter might act indirectly, affecting the normal cells and reducing their ability to inhibit growth of the *ras* transformants. To distinguish between these possibilities, we infected cells with the *ras* or the *myc* virus and split them 2 days later into two groups, one being transferred to medium with G418 alone, the other to medium containing G418 plus TPA. In this way, all adjacent normal cells were killed off, and the oncogene-bearing cells could grow up into isolated colonies.

As is clear from Fig. 3, within 1 week after plating, there was a strong effect of TPA on the size, but not the number, of '*ras* cell' colonies in a culture dish. When cells were trypsinized and counted, 10 times more cells were found in the presence of TPA than in its absence. No such difference could be detected in either the control cells or the *myc*-bearing cells. We conclude that at least part of the synergism between TPA and the *ras* oncogene derives from direct effects on the *ras*-bearing cells. While TPA might be able to affect adjacent normal cells, such effects do not need to be invoked to explain our results.

Initiated cells *in vivo* are induced to grow out into papillomas by the continued application of TPA^{1,5,6} and this effect can be blocked by the concomitant application of retinoic acid¹³. As Table 1 shows, retinoic acid exerts an analogous activity in our system, in that it can effectively block the TPA-dependent outgrowth of '*ras*' foci. Thus, TPA and retinoic acid seem to interact similarly *in vivo*, in the promotion and repression of papilloma formation, and *in vitro*, in the stimulation and suppression of growth of *ras*-transformed cells.

Promotion of papillomas can be resolved into two steps^{14,15}. First-stage promotion can be brought about by a single application of TPA, while second-stage promotion requires prolonged applications of either TPA or 'second-stage promoters', such as 12-*O*-retinoylphorbol-13-acetate (RPA) or mezerein, which are either not active or very weakly active in the first stage^{14,15}. These compounds, when tested in our system, were found to be about

Table 1 Effects of various chemicals on focus formation of REFs bearing *ras* oncogene

Chemical	Number of foci per dish
—	0
TPA (10 ng ml^{-1})	55
TPA + RA ($10 \mu\text{M}$)	0
TPA + RA ($1 \mu\text{M}$)	0
TPA + RA ($0.1 \mu\text{M}$)	5
TPA + RA ($0.01 \mu\text{M}$)	45
RPA (100 ng ml^{-1})	60
RPA (10 ng ml^{-1})	40
Mezerein (100 ng ml^{-1})	45
Mezerein (10 ng ml^{-1})	10
4 α -PDD (100 ng ml^{-1})	0
4 α -PDD (10 ng ml^{-1})	0

REFs were infected as described in Fig. 1 legend and after 48 h re-plated (1:10 split) into either normal medium or medium containing the various chemicals; 10 days later, the dishes were stained and foci counted. Two independent dishes were counted for each value shown. TPA and retinoic acid (RA) were purchased from Sigma; RPA, mezerein and 4 α -PDD from LC Services Corp., Woburn, Massachusetts.

as active as TPA (Table 1); this suggests that introduction of an activated *ras* oncogene might bypass the requirements for first-stage promotion and allow these cells to respond directly to second-stage promoters. Another phorbol ester derivative, 4 α -phorbol-12,13-didecanoate (4 α -PDD), which has been shown previously to be inactive both *in vivo* and *in vitro*¹⁶, was also tested in our system and found to have no activity (Table 1).

Activated *ras* and *myc* oncogenes have been found in a large variety of tumours, both of spontaneous origin and chemically induced, and it is assumed that activation of these genes has a causal role in tumour development¹⁷. The present work provides a direct and quantitative demonstration that introduction of an activated *ras*—but not *myc*—oncogene into primary cells causes these cells to respond to a promoter in the manner of an initiated cell. This provides an *in vitro* parallel with work from other laboratories which suggests that *ras* activation occurs at very early times during initiation/promotion of carcinogenesis *in vivo* and may even coincide with the initiating event^{18–21}.

Others have recently reported that TPA is able to act synergistically with *myc*²². This observation is only in apparent contrast to ours as these workers used TPA concentrations that were 1,000-fold higher than those used in the present study and they

detected foci of *myc* transfectants in immortalized cells in the presence of TPA only after 6 weeks, in contrast to the 1-week lag period reported here.

TPA, like the *myc* oncogene, is able to collaborate with a *ras* oncogene to allow focal overgrowth in monolayer culture. In this limited sense, TPA acts analogously to *myc*, achieving epigenetically the same effects as those of a genetically activated *myc* gene. We note that TPA has been found to induce expression of the normal cellular *myc* gene²³. This induction may represent the mechanistic basis by which TPA is able to mimic *myc* in the present experimental model.

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C_{μ} -containing transcripts initiate heterogeneously within the *IgH* enhancer region and contain a novel 5'-nontranslatable exon

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Transcriptional competence of the immunoglobulin heavy-chain locus (*IgH*) is established at an early stage of lymphoid cell development, leading to the appearance of RNA components, previously called C_{μ} RNA¹ or sterile- μ RNA², which contain constant-region sequences but lack variable-region sequences. These components are of two types: those which initiate in the *D* region of alleles that have undergone *DJ_H* (diversity-joining region) rearrangement (D_{μ} transcripts) and those which initiate within the *J_H-C_μ* intron (hereafter termed I_{μ} transcripts)^{3,4}. In pre-B and early B cells, D_{μ} and I_{μ} transcripts are nearly as abundant as the messenger RNA encoding μ heavy chain^{2,3,5}. The D_{μ} transcripts are spliced into RNAs containing *D*, *J_H* and C_{μ} sequences, and in some, but not all, cases these RNAs are translated into D_{μ} proteins⁴. To establish whether the I_{μ} transcripts have any translational potential and to elucidate the structure of their promoter region, we have determined their transcription initiation sites and their mode of splicing. As reported here, by using sequence

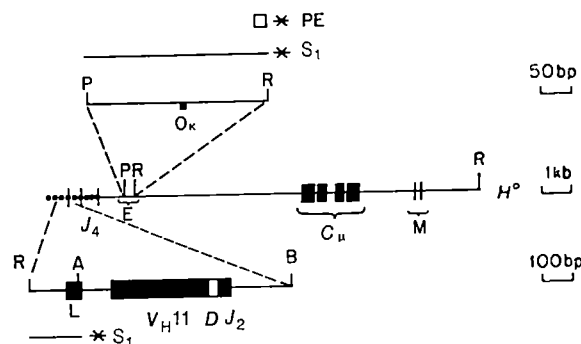


Fig. 1 Schematic diagram of mouse immunoglobulin heavy chain genes and relevant probes. The germline (H^+) region that is common to both alleles of the lymphoma 38C-13 (ref. 3) is shown in the middle of the figure. The upper expansion of the enhancer (E) region shows the location of the octanucleotide (O_8) oriented as in V_{κ} genes. The box to the left of PE (primer extension) indicates the binding position of the 21-mer oligonucleotide. The *PvuII-EcoRI* S_1 probe was isolated from an *Sp6* subclone containing a 700-base pair (bp) *XbaI-EcoRI* insert. The lower expansion shows the 5' region of the productive allele. The *EcoRI-AhaIII* S_1 probe was isolated from subclone p38CV which contains the *IcoRI-BamHI* insert shown. Abbreviations of restriction sites: P, *PvuII*; R, *EcoRI*; B, *BamHI*; A, *AhaIII*. (Not all sites are shown.)

analysis of cloned I_{μ} complementary DNAs, primer extension and S_1 nuclease mapping, we have found that these transcripts have remarkable 5' heterogeneity: there are more than five distinct start sites spanning a region of 44 nucleotides that is located downstream of an octanucleotide found in all variable-region promoters. Such imprecise initiation may result from the lack of a well-defined TATAA motif and the unusual proximity of the octanucleotide to the enhancer region. Approximately 700 nucleotides downstream from these initiation sites, a cryptic splice site is used to create a nontranslatable exon ('nontron') which is joined to the C_{μ} domain. The properties of the nontron may be important for the mechanism of allelic exclusion.

Cloned cDNAs representative of I_{μ} transcripts were obtained from a λ gt10 cDNA library made from RNA of the mouse lymphoma 38C-13. This lymphoma contains cytoplasmic I_{μ} RNA components, presumably transcribed from both productive (H^+) and nonproductive (H^-) alleles, which can be readily distinguished from the mRNA encoding the complete μ heavy chain by their ability to hybridize with a probe specific for a 5' portion of the *J_H-C_μ* intron³. On screening the library with such a probe (IVS₁, ref. 3), we obtained 12 positive clones out of approximately 6×10^5 plaques. The two longest cDNA inserts were purified, subcloned into pUC plasmids, and sequenced. The sequence of their 5' ends mapped within the 0.32-kilobase (kb) *PvuII-EcoRI* fragment previously shown to contain the 5' boundary of some of the C_{μ} RNA components¹ as well as the heavy-chain enhancer element^{6,7}.

To localize precisely the sites at which the I_{μ} transcripts initiate, we used both primer extension and S_1 nuclease mapping (Fig. 1). For primer extension of 38C-13 cytoplasmic RNA, we used a 21-mer oligonucleotide representing a sequence near the 3' boundary of the enhancer region. Several products extending 37-80 nucleotides upstream of the primer were observed (Fig. 2a), suggesting the existence of either a multiplicity of transcriptional start sites or a series of reverse transcriptase-dependent artefactual stop sites. S_1 nuclease analysis using a single-stranded *PvuII-EcoRI* fragment allowed us to distinguish between these two possibilities. As a control, we simultaneously determined the start site of the 38C-13 μ mRNA, using the same RNA preparation and an *EcoRI-AhaIII* fragment spanning the leader exon of the productive H^+ gene (Fig. 1). The results of this analysis were clear-cut: the μ mRNA initiates at a unique site (Fig. 2c, lanes 3, 4), whereas the initiation sites of the I_{μ} transcripts are indeed heterogeneous (Fig. 2b, lane 2), corresponding exactly to those seen by the primer extension assay. A similar result was obtained with RNA from C2 cells, a

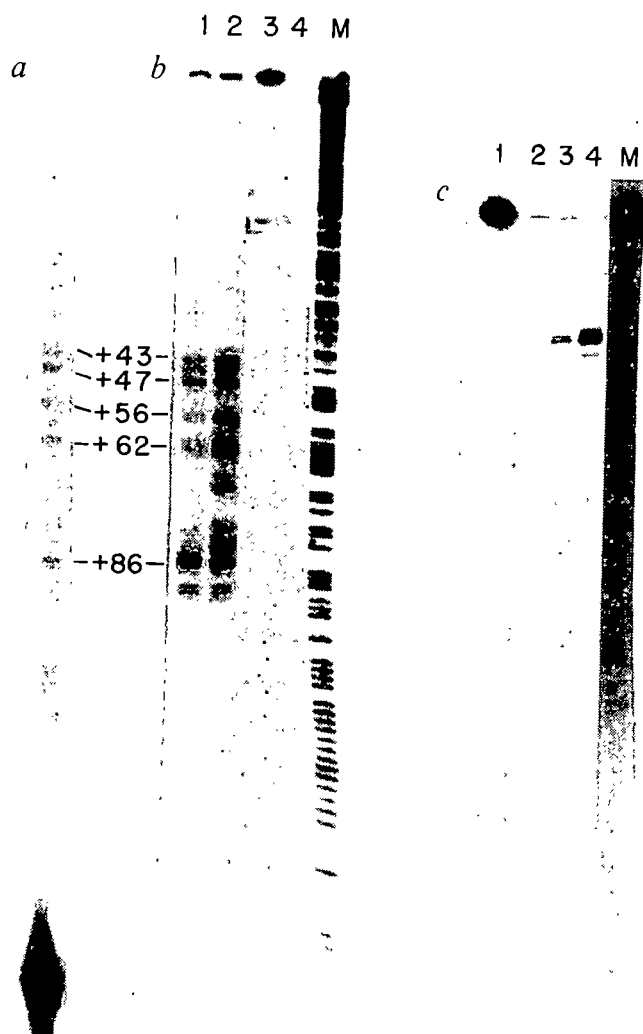


Fig. 2 Mapping of transcription initiation sites. **a**, Primer extension. 50 ng of 21-mer labelled with [γ -³²P]dATP was hybridized to 10 µg of 38C cytoplasmic poly(A)⁺ RNA for 33 h at 37 °C in 20 µl of a solution containing 80% formamide³¹ and 21 µg of poly(A)⁺ cytoplasmic carrier RNA obtained from MPC 11 myeloma cells. Most of the unannealed primer was removed by oligo(dT)-cellulose chromatography, and the remaining material extended using avian myeloblastosis virus reverse transcriptase. Products were loaded onto a 10% polyacrylamide/7 M urea thickness-gradient gel. Numbering indicates the distance (in nucleotides) downstream from the first nucleotide of the conserved octanucleotide. The strong band at the bottom is non-extended primer. **b**, S₁ protection assay of *I*_μ transcripts. RNA samples were annealed to a gel-separated minus-strand probe (upper expansion, Fig. 1) for 3 h at 40 °C, then the samples were diluted and treated for 37 min at 19 °C with 4,000 units of S₁ nuclease³². Lane 1, 20 µg C2 cytoplasmic poly(A)⁺ RNA; lane 2, 20 µg 38C cytoplasmic poly(A)⁺ RNA; lanes 3, 4, no RNA (S₁ nuclease treatment omitted for lane 3). **c**, S₁ protection assay of *H*⁺ transcripts. Same general protocol as for **b**. The minus-strand probe is shown in the lower extension of Fig. 1. Lanes 1 and 2, 1 and 5 µg of carrier RNA, respectively (S₁ nuclease treatment omitted for lane 1); lane 3, 1 µg 38C cytoplasmic poly(A)⁺ RNA; lane 4, 5 µg 38C cytoplasmic poly(A)⁺ RNA. M, G + A sequencing reactions of the appropriate probe plus strands, one of the three such sequencing reactions used as markers. Transcription initiation sites were determined from a comparison of S₁ nuclease-resistant fragments and marker lanes, applying the 4.5-nucleotide correction factor established in previous experiments³³.

hybridoma derivative of 38C-13 which contains only the nonproductive (*H*⁻) allele³ (Fig. 2b, lane 1). There appears to be a difference in the relative abundance of the various fragments protected by 38C and C2 RNA, suggesting a possible difference in the relative utilization of the different *I*_μ start sites in these two cells. Whether this difference is related to a difference in *I*_μ transcription on the *H*⁺ and *H*⁻ alleles remains to be established.

The *I*_μ start sites are located (Fig. 3a) 43–86 nucleotides downstream of a highly conserved^{8,9} octanucleotide (ATTTGCAT) which is found in all *V*_κ genes and, in inverse orientation, in all *V*_H genes about 60–80 nucleotides upstream of the cap site. This octanucleotide element is apparently essential for the transcriptional activity of these genes^{9,10}. *In vivo* footprinting assays¹¹ have indicated that almost all tissue-specific protein binding in the enhancer region occurs upstream of this octanucleotide (Fig. 3a), suggesting that this region may contain binding sites for factors that are important for the generation of *I*_μ transcripts. Comparing the position of the octanucleotide implicated in *I*_μ transcription with that of an octanucleotide brought upstream of the enhancer by the productive rearrangement of a *V*_H gene, we note that the *I*_μ octanucleotide is located on the opposite side of these binding sites and in inverse orientation. Thus, the polarity relative to the binding sites is the same for both *I*_μ and *H*⁺ octanucleotides.

The 38C *H*⁺ gene promoter has a TATAA box correctly spaced upstream of the unique initiation site (Fig. 3b), whereas for the *I*_μ transcripts there is no unique, well-defined TATAA box. Initiation heterogeneity similar to that observed for *I*_μ transcription has been observed at sites adjacent to the simian virus 40 (SV40) and polyoma enhancers^{12,13}. The viral promoters responsible for these transcripts also lack TATAA box homologies. To date, very few cellular genes with this extent of

heterogeneity have been described, although one interesting exception is the hydroxymethyl glutaryl coenzyme A reductase gene, which initiates heterogeneously in a G + C-rich region that lacks a TATAA box¹⁴. However, as several other genes, including even some *V*_H and *V*_κ genes, can initiate precisely despite the lack of a good TATAA motif (see refs 15 and 16 for examples), it is clear that the absence of this motif does not necessarily engender transcriptional heterogeneity. Perhaps the unusual proximity of the octanucleotide to the major protein-binding region of the enhancer also contributes to the imprecise initiation of *I*_μ transcripts.

Knowledge of the cDNA sequences allowed us to identify the 5' splice site used in the processing of the *I*_μ transcripts. The junction between the *J*_H-*C*_μ intron sequence and the *C*_μ1 exon is the same in both cDNA sequences; it defines a splice site 735 nucleotides downstream from the most 5' of the transcriptional start sites (Fig. 3c). The sequences flanking this splice site, which is normally not recognized in the splicing of pre-*μ* mRNA or *D*_μ transcripts, conform reasonably well to the consensus for 5' splice sites¹⁷. Utilization of this cryptic splice site creates a large exon that contains over 35 termination codons in the three reading frames (Fig. 4). Indeed, in the frame that encodes the *C*_μ protein, there are more than 10 stop codons distributed over the entire exon segment, and utilization of the first non-blocked initiation codon would eliminate 25 amino acids from the *C*_μ1 domain. Moreover, according to present ideas about translation initiation¹⁸, such an exceptionally long nontranslatable 5' exon (nontron), with at least 12 AUG codons, should greatly reduce the efficiency with which *I*_μ transcripts can be translated into any protein with *C*_μ determinants. Thus, it is now evident why such proteins are not detected in cells that are demonstrably producing *I*_μ transcripts¹⁹.

A matrix-plot comparison of the mouse and human *J*_H-*C*_μ intron sequences revealed a remarkable (for intron sequences) conservation of the 5' portion of this intron²⁰. The conserved segment not only embraces the enhancer region, including a 7/8 octanucleotide homologue, but in fact extends slightly beyond the 3' border of the nontron and includes its splice junction and over 40 stop codons. The fact that both the cryptic splice site and the multiplicity of stop codons are evolutionarily conserved suggests that the nontron may be of functional significance. What could be the significance of an element that prevents *C*_μ translation? One possibility which we find attractive is related to mechanistic models of allelic exclusion^{21–23}. An important attribute of these models is a feedback system which causes the

Fig. 3 Sequence characteristics of I_μ transcripts. *a*, A 298-nucleotide sequence of the *IgH* enhancer region (as listed in GenBank and confirmed by us) showing the transcription initiation sites (*). The *PvuII* site corresponds to that shown in Fig. 1. The octanucleotide is boxed; ▼ indicates the nucleotide where the longest cDNA begins; ○ and ●, respectively, indicate protection from, and enhancement of, reactivity with dimethyl sulphate by lymphocyte-specific factors¹¹. *b*, Sequence of the 5' region of the 38C H^+ gene (downstream of the *EcoRI* site, determined by chemical cleavage; upstream, taken from the sequence³³ of a closely related gene, V_{H11}) showing the initiation site (*), as well as the octanucleotide and TATAA motifs (boxed). Horizontal arrows under the octanucleotides emphasize the inverted polarity of these sequences in I_μ and V_H promoters. *c*, Sequence spanning the site (/) at which the 5' portion of the I_μ transcript is spliced to the C_μ exon, as determined from the cDNA sequence. The sequence of this region agrees with that listed in GenBank.

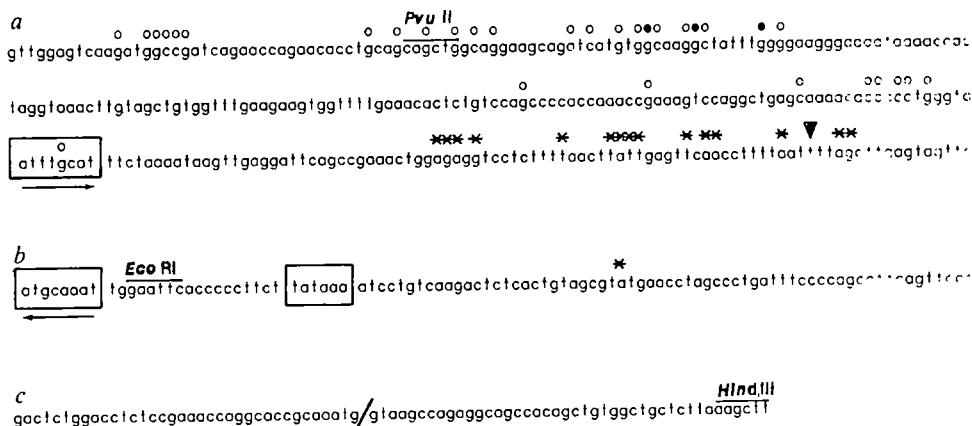
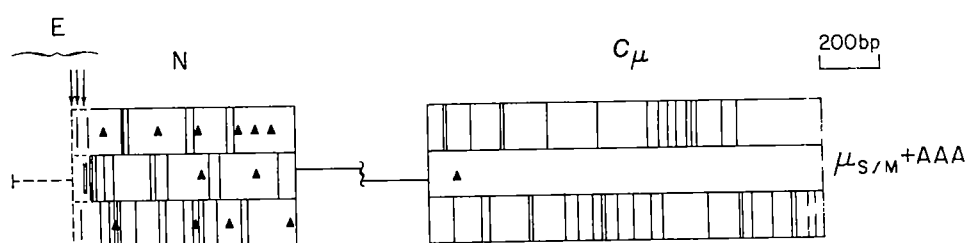


Fig. 4 Schematic diagram of the processed portions of I_μ transcripts. N, nontron; E, enhancer region; ↓↓, initiation sites; C_μ , entire C_μ region, which is joined to either a secretory (μ_S) or a membrane-associated (μ_M) polyadenylated terminus. Reading frames are aligned according to the splice junction shown in Fig. 3c. ▲, Location of AUG codons in the nontron (the first AUG in the C_μ coding frame is also indicated); vertical bars represent stop codons.



developing B lymphocyte to cease rearranging its immunoglobulin genes when a productive rearrangement has been made. There are two stages at which the cell apparently recognizes such feedback signals: first, when a heavy-chain gene has been productively rearranged, and later when a productive light-chain rearrangement occurs. While the second signal could be related to the appearance of a completely assembled immunoglobulin on the cell surface, the first would presumably result from the appearance of intracellular μ heavy chain. If the C_μ region were the critical determinant for this feedback signal, then any translation products of the I_μ transcripts could repress the rearrangement of heavy-chain genes prior to the formation of a productive allele. Thus, we propose that the nontron prevents the generation of a feedback signal which would prematurely curtail heavy-chain gene rearrangement. We are implicitly assuming here that early transcriptional activity at the heavy-chain locus is essential for, or at least an obligatory concomitant of, the rearrangement mechanism. The fact that C_κ (refs 24, 25), V_λ (ref. 26) and some V_H (ref. 27) regions are transcriptionally active prior to rearrangement lends support to this notion.

If our proposal is correct and any C_μ -containing polypeptide can serve as a feedback signal, we must presume that the so-called D_μ proteins, which are produced when the 5'-flanking regions of certain D elements are joined in the proper reading frame to a J element, would also inhibit further rearrangement. Although examples of Abelson virus-transformed pre-B-cell lines that produce D_μ proteins and are still capable of undergoing VD rearrangement have been reported⁴, more recent findings²⁸ indicate that such rearrangement occurs only when the level of D_μ protein is very low, and that high levels of D_μ protein may, in fact, be inhibitory for recombination. The I_μ RNAs are more abundant than D_μ RNA (ref. 3 and our unpublished observations) and are generated over a longer time span since their production is independent of the rearrangement status of the H alleles. Hence, if there were translation products of I_μ RNAs, they would probably be at sufficiently high con-

centrations to repress recombination. We also find it noteworthy that for most H^+ loci in which the D segment can be unambiguously identified, the $D-J$ join is in a frame that could not have previously produced a D_μ protein^{29,30}. Thus, there may actually be an inverse correlation between the ability to produce D_μ protein and the subsequent possession of a completely rearranged H allele.

Irrespective of its implications for allelic exclusion, the properties of the nontron may represent a means of accommodating a possible requirement for transcriptional competence at the J_H-C_μ locus while avoiding the deleterious consequences of a protein potentially encoded by the transcription product.

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Resistance to β -lactam antibiotics by re-modelling the active site of an *E. coli* penicillin-binding protein

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The β -lactam antibiotics kill bacteria by inhibiting a set of penicillin-binding proteins (PBPs) that catalyse the final stages of peptidoglycan synthesis^{1,2}. In some bacteria the development of intrinsic resistance to β -lactam antibiotics by the reduction in the affinity of PBPs causes serious clinical problems^{1,3-11}. The introduction of β -lactam antibiotics that are resistant to hydrolysis by β -lactamases may also result in the emergence of intrinsic resistance among the Enterobacteriaceae¹. The clinical problems that would arise from the emergence of resistant PBPs in enterobacteria have led us to examine the ease with which *Escherichia coli* can gain resistance to β -lactams by the production of altered PBPs. The development of resistant PBPs also provides an interesting example of enzyme evolution, since it requires a subtle re-modelling of the enzyme active centre so that it retains affinity for its peptide substrate but excludes the structurally analogous^{2,12,13} β -lactam antibiotics. We show here that only four amino-acid substitutions need to be introduced into PBP 3 of *E. coli* to produce a strain possessing substantial levels of resistance to a wide variety of cephalosporins. We also show that transfer of the gene encoding the resistant PBP 3 from the chromosome to a plasmid could result in the spread of intrinsic resistance not only to other strains of *E. coli* but also to other enterobacterial species.

Most β -lactam antibiotics in clinical use kill *E. coli* at their minimum inhibitory concentrations by inactivation of PBP 3, resulting in the inhibition of cell division and the growth of the bacteria into filamentous cells^{1,14}. Many of these antibiotics (for example, cephalixin, cefuroxime, third-generation cephalosporins, mezlocillin, piperacillin and monobactams¹⁵) show much greater affinity for PBP 3 than for the other essential PBPs, and therefore kill *E. coli* over a wide concentration range exclusively by inactivation of PBP 3¹. Substantial levels of resistance to these β -lactams could therefore be achieved by the development of a form of PBP 3 that has reduced affinity for the antibiotics. Amino-acid substitutions that allow PBP 3 to discriminate between the binding of cephalixin and its normal substrate are, however, very rare and result in only a low level of resistance¹⁶. Mutants with high levels of resistance require multiple amino-acid substitutions in PBP 3 which re-model the active centre to reduce its affinity for β -lactams without impairing its ability to process the normal substrate. We have followed

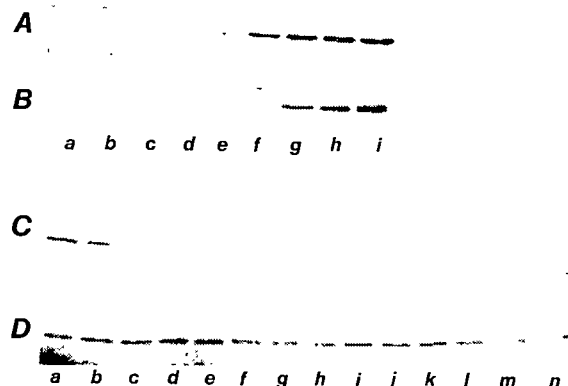


Fig. 1 Affinity of PBP 3 of the fourth-level mutant (SP1124) for benzylpenicillin and cephalixin²⁶. Increasing concentrations of ³H-benzylpenicillin (21 Ci mmol⁻¹; a gift of Merck Sharp and Dohme) were incubated for 10 min at 30 °C with washed cell envelopes prepared from *E. coli* expressing wild-type PBP 3 (strain C600pPH105) (A) or the resistant PBP 3 of the fourth-generation mutant (strain SP1124pPH105pbpB^R) (B). The reactions were terminated and the PBPs fractionated on a 10% SDS polyacrylamide gel as described elsewhere²⁶. The levels of ³H-benzylpenicillin bound to the PBPs were determined by fluorography. Only the section of the fluorograph showing PBP 3 is illustrated. ³H-benzylpenicillin concentrations (μ g ml⁻¹): a, 0.06; b, 0.12; c, 0.25; d, 0.5; e, 1; f, 2; g, 4; h, 8; i, 16. A measure of the affinity of the PBPs for cephalixin was obtained by incubating washed cell envelopes of strain C600pPH105 (C) or SP1124pPH105pbpB^R (D) with increasing concentrations of cephalixin for 10 min at 30 °C and then adding ³H-benzylpenicillin (30 μ g ml⁻¹ final concentration) for a further 10 min to label the PBPs that are not complexed with cephalixin²⁶. The PBPs were separated and visualized as above. Only the section of the fluorograph showing PBP 3 is illustrated. Cephalixin concentrations (mg ml⁻¹): a, 0; b, 0.015; c, 0.03; d, 0.06; e, 0.12; f, 0.24; g, 0.47; h, 0.95; i, 1.9; j, 3.8; k, 7.5; l, 15; m and n, 30.

the evolution of *E. coli* PBP 3 during selection for increasing levels of resistance to cephalosporins.

The most resistant of the cephalixin-resistant mutants obtained in our previous study¹⁶ (SP1091; Table 1) was mutagenized separately with ethyl methane sulphonate, ultraviolet irradiation and nitroquinoline *N*-oxide, and second-level mutants showing increased levels of resistance to cephalixin were selected at 37 °C. Those mutants in which the increased resistance was due to a further reduction in the affinity of PBP 3 for cephalixin were distinguished from the majority of the mutants in which resistance was due to decreased outer membrane permeability¹⁷ or increased expression of the chromosomal β -lactamase¹⁸, by the phage P1-mediated co-transduction of the resistance with the *leu* genes which map very close to the PBP 3 gene (*pbpB*)¹⁹. However, second-level PBP 3 mutants that showed a substantial increase in resistance could not be obtained from any of the mutagenized cultures and the best mutants obtained showed only a slight increase in cephalixin resistance. The decrease in the affinity of PBP 3 for cephalixin in SP1091 resulted in a decrease in its thermostability so that the strain grew well at 37 °C but filamented and died at 43 °C¹⁶. The second-level mutants had regained the normal thermostability of PBP 3 and the ability to form colonies at 43 °C.

One of the second-level mutants (SP1096, a transductant carrying the mutant *pbpB* gene in an unmutagenized background) was treated with each of the mutagens and selection was applied for a further increase in cephalixin resistance; those third-level mutants in which the increased resistance was due to PBP 3 changes were identified. Repeated attempts to obtain third-level mutants produced only one isolate (SP1097), which showed a further substantial increase in PBP 3-mediated cephalixin resistance. The decrease in affinity of PBP 3 of SP1097 was accompanied by decreased thermostability and the

Table 1 Properties of a series of PBP 3 mutants with increasing levels of resistance to cephalosporins

Strain	Minimum inhibitory concentration ($\mu\text{g ml}^{-1}$) of														Growth at 43 °C
	Clxn	Cdrx	Crln	Cthn	Crxm	Cmdl	Ctxm	Ctaz	Cprz	Ampn	Pipn	Mzln	Ticn	Aztr	
C600 (parent)	6	10	20	5	5	1	0.1	0.2	0.2	5	2	2	5	0.1	Yes
SP1091 (first level)	50	100	200	100	100	2	0.1	0.1	0.1	1	0.5	0.5	0.5	0.05	No
SP1096 (second level)	60	100	100	20	10	5	0.1	0.1	0.5	10	2	2	1	0.1	Yes
SP1097 (third level)	500	400	1,000	200	200	40	1	1	0.5	5	1	1	1	0.05	No
SP1124 (fourth level)	500	400	2,000	200	500	40	2	2	1	5	5	2	2	0.1	Yes

The isolation of the first-level mutant, SP1091, and the methods for identifying *pbpB* mutants, have been described elsewhere¹⁶. The mutants with increasing levels of resistance to cephalosporins were isolated after mutagenesis with ethyl methane sulphonate²⁰, ultraviolet irradiation²¹ or nitroquinoline *N*-oxide²¹ as described in the text. Determination of minimal inhibitory concentrations: 10- μl amounts of a 10^{-5} dilution of overnight cultures of the mutants and the parent strain (~ 150 colony-forming units) were spotted on L-agar containing a range of concentrations of the antibiotics. The plates were incubated at 37 °C for 24 h and the minimum inhibitory concentration was the lowest concentration of antibiotic that prevented bacterial growth. A similar number of cells were plated on L-agar at 43 °C to test the ability of the mutants to grow at this temperature. Abbreviations for cephalosporins: Clxn, cephalixin; Cdrx, cefadroxil; Crln, cephradine; Cthn, cephalothin; Crxm, cefuroxime; Cmdl, cefamandole; Ctxm, cefotaxime; Ctaz, ceftazidime; Cprz, cefoperazone. Abbreviations for penicillins: Ampn, ampicillin; Pipn, piperacillin; Mzln, mezlocillin; Ticn, ticarcillin. Aztr is aztreonam, a monobactam¹⁵.

Table 2 Nucleotide changes in the *pbpB* gene, and the predicted amino-acid substitutions in PBP 3, of a series of mutants showing increasing levels of resistance to cephalosporins

Strain	Mutagen used for isolation	Nucleotide change in <i>pbpB</i>	Amino-acid substitution in PBP 3
SP1091 (first level)	Ethyl methane sulphonate	AAC to AGC	Asn 361 to Ser
SP1096 (second level)	Ethyl methane sulphonate	GTT to ATT	Val 530 to Ile
SP1097 (third level)	Ultraviolet irradiation	TAC to CTC	Tyr 541 to Leu
SP1124 (fourth level)	Ethyl methane sulphonate	GAA to AAA	Glu 349 to Lys

The wild-type *pbpB* gene was cloned into the pSC101-derived vector, pLG339, to produce pPH105²². The mutations in the *pbpB* gene of each of the mutants were transferred into pPH105 *in vivo* by homogenization as described previously¹⁶. The wild-type and mutant *pbpB* genes were cloned from these plasmids into phage M13 mp8/9 vectors²³ and were dideoxy-sequenced²⁴ on both strands using a set of oligonucleotide primers complementary to regions along each strand of the *pbpB* gene as described previously¹⁶. The nucleotide sequence of the wild-type *pbpB* gene has been determined by Nakamura *et al.*²⁵. The nucleotide changes shown are the new mutations that have occurred at each level; in addition, the *pbpB* gene of the mutants contained the mutations they obtained at the earlier levels.

Table 3 Conferral of cephalosporin resistance on enterobacteria by the introduction of a plasmid expressing a cephalosporin-resistant form of PBP 3

Strain	Minimum inhibitory concentrations ($\mu\text{g ml}^{-1}$) of:						
	Clxn	Crln	Cthn	Crxm	Cmdl	Ctxm	Cprz
<i>E. coli</i> pPH105	6	20	5	5	1	0.1	0.2
<i>E. coli</i> pPH105 <i>pbpB</i> ^{rs}	500	2,000	200	500	50	2	1
<i>S. typhimurium</i> pPH105	5	20	1	2	<0.2	0.1	0.5
<i>S. typhimurium</i> pPH105 <i>pbpB</i> ^{rs}	500	500	20	100	5	0.5	2
<i>K. pneumoniae</i> pPH105	5	10	2	2	0.5	0.05	0.5
<i>K. pneumoniae</i> pPH105 <i>pbpB</i> ^{rs}	500	1,000	50	100	50	0.5	5

pPH105, expressing wild-type PBP 3, and pPH105*pbpB*^{rs}, expressing the resistant PBP 3 of the fourth-level mutant SP1124, were introduced by transformation into *E. coli* C600, *S. typhimurium* LR5000 and *Klebsiella pneumoniae* UNF122. The abbreviations for antibiotics and the method for measuring the minimum inhibitory concentrations are as in Table 1.

strain filamented at 43 °C. The increased resistance of strain SP1097 was due to a double nucleotide change in *pbpB* (Table 2). Presumably SP1097 was obtained at such a low frequency because there were no single-nucleotide changes in the *pbpB* gene of the second-level mutant that produced the desired phenotype.

SP1097 lysed when grown in the presence of its minimum inhibitory concentration of cephalixin, indicating that the affinity of PBP 3 had been reduced to the extent that killing was now occurring by the inactivation of PBP 1A/1B rather than PBP 3. The mutant had therefore achieved the maximum level of resistance to cephalixin that could be achieved by a reduction in the affinity of PBP 3 and had also gained close to the maximum possible levels of cross-resistance to a number of other cephalo-

sporins (such as cefadroxil, cephradine, cefuroxime and cephalothin). The mutant had, however, not achieved the maximum possible levels of resistance to third-generation cephalosporins and further attempts to re-model the active site of PBP 3 to give resistance to all of those cephalosporins that kill by inactivation of PBP 3 were carried out by selecting for increased resistance to cefoperazone, ceftazidime and cefotaxime. The fourth-level mutants obtained by selecting for resistance to the above compounds had only a slight further increase in their resistance to cephalosporins and had simultaneously regained the ability to form colonies at 43 °C. Attempts to produce fifth-level mutants by selecting for additional resistance to third-generation cephalosporins have been unsuccessful.

The properties of the series of mutants are shown in Table 1, while Table 2 shows the nucleotide substitutions that have occurred in the *pbpB* gene at each level of selection. Interestingly, the evolution of PBP 3 in this series of mutants has occurred by amino-acid substitutions that alternately reduce the affinity of the enzyme for cephalosporins but de-stabilize the enzyme, and those that have little or no effect on affinity but which re-stabilize the enzyme.

The fourth-level mutant (SP1124) showed the maximum levels of resistance to almost all cephalosporins that can be achieved by reduction of the affinity of PBP 3 (Table 1). The increase in resistance that has been gained in the latter mutant by four amino-acid substitutions in PBP 3 would probably be sufficient to prevent successful therapy by most cephalosporins.

SP1124 showed cross-resistance to those cephalosporins that kill by inactivation of PBP 3 but not to any of the penicillins or monobactams that were tested. As expected, the affinity of strain SP1124 PBP 3 for benzylpenicillin was only slightly reduced from that of the parent strain (C600), whereas its affinity for cephalixin was greatly reduced (Fig. 1). It might be expected, *a priori*, that penicillins, monobactams and cephalosporins would have a substantial degree of overlap in their binding sites on the enzyme and it is surprising that the re-modelling of PBP 3 to reduce its affinity for cephalosporins has had no apparent effect on the binding of the penicillins or monobactams. This suggests that there may, in fact, be little overlap between the binding sites for cephalosporins and the other classes of β -lactam antibiotics. Further evolution of the active site of PBP 3 to exclude penicillins and monobactams as well as cephalosporins could probably be achieved, but we have not attempted to do this for safety reasons.

A plasmid that expressed the *pbpB* gene of the fourth-level mutant could transform a sensitive strain of *E. coli*, *Salmonella typhimurium* or *Klebsiella pneumoniae* to broad-spectrum cephalosporin resistance (Table 3). If resistant forms of PBP 3 evolve in clinical isolates, the transfer of the altered PBP gene to a plasmid could therefore result in the infectious spread of resistance through *E. coli* and other species of the Enterobacteriaceae. The consequences of such events for the treatment of enterobacterial infections with β -lactam antibiotics would be considerable.

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The immunodominant site of a synthetic immunogen has a conformational preference in water for a type-II reverse turn

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Many short synthetic peptides have now been shown to induce antibodies reactive with their cognate sequences in the intact folded protein¹⁻³. Aside from the usefulness of such antibodies as site-specific reagents, the frequency with which this recognition occurs has raised several theoretical issues, the central one being that of how an antibody to a short synthetic peptide, which represents one of the most disordered states of a site in a protein, can react with the more ordered version of the same sequence in the folded protein. This apparent paradox can be resolved if the target site on the protein approaches disorder or if the peptide in solution or on a carrier adopts, with significant frequency, a conformation compatible with that of the cognate site in the protein. Various studies already suggest that antigenic sites in proteins correspond to regions of high atomic mobility^{1,9-15}. We now show, using high-field nuclear magnetic resonance (NMR) spectroscopy, that a nonapeptide selected by several monoclonal antibodies as the immunodominant site of a 36-amino-acid immunogen (residues 75-110 of influenza virus haemagglutinin^{16,17}) adopts a highly populated type-II reverse-turn conformation in water. This suggests that in this case the antibodies have selected a sequence possessing a conformational preference. Apart from helping us to understand immunological recognition, anti-peptide antibodies may provide reagents of sufficient precision for an immunological approach to the problem of protein folding¹⁸⁻²³.

^1H -NMR spectroscopy provides a powerful method for determining molecular conformation in solution. In the present work, resonances were assigned to specific protons in the peptide using a combination of one- and two-dimensional methods. The conformation of the molecule was then examined by measuring nuclear Overhauser effects (NOEs), coupling constants and temperature and pH dependence of the NMR spectrum. The assignment procedure is illustrated in Fig. 1, which shows part of a two-dimensional scalar-correlated (COSY)²⁴⁻²⁶ spectrum. The cross-peaks establish unambiguously the coupling patterns (connectivities) for the resonances of each type of amino acid. Assignment to specific amino acids was based primarily on the observation of NOEs between resonances of sequentially adjacent amino acids. A detailed description of the assignments will be given elsewhere.

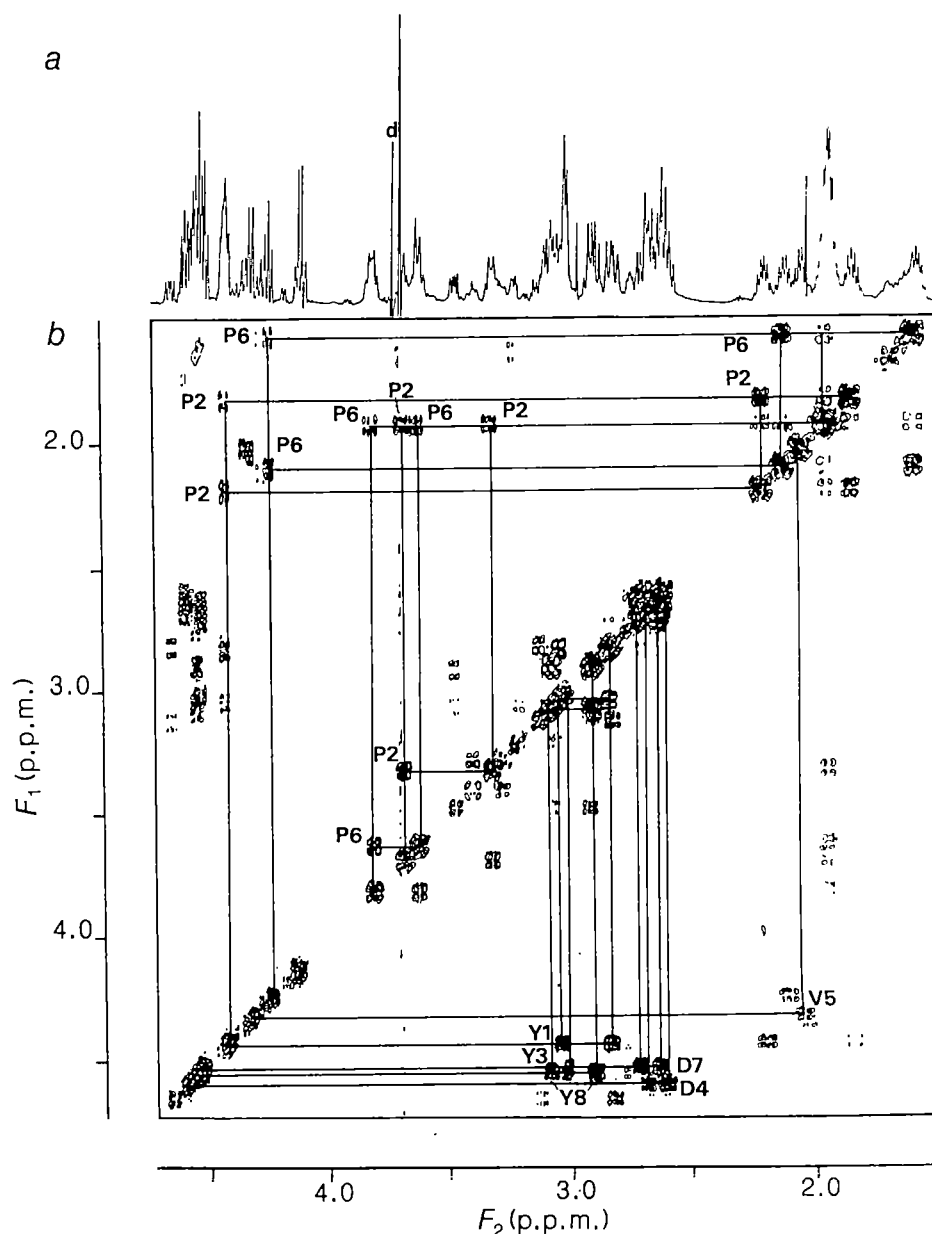
The NMR spectra of the peptide Tyr-Pro-Tyr-Asp-Val-Pro-Asp-Tyr-Ala are complicated by *cis-trans* isomerism²⁷ about the Tyr 1-Pro 2 peptide bond. This results in two distinct forms of the peptide which interconvert slowly on the NMR timescale. The relative intensities of peaks in the spectrum show that the two isomeric forms are present in the approximate *trans:cis* ratio of 2:1. This ratio appears to be little influenced by pH changes. Only the *trans* form appears to have demonstrable structure in water solution and thus is the subject of most of the following discussion.

The resonances of the exchangeable amide protons are absent from spectra of the peptide in D_2O . In H_2O , 12 amide proton resonances are observed, 6 from the *trans* and 6 from the *cis* form of the peptide (no NH proton resonances are expected for the N-terminal tyrosine or, of course, for the two prolines). The NH proton resonances were assigned from a COSY spectrum of the peptide in water solution (Fig. 2). Table 1 lists the assignments of the αCH and NH proton resonances. Unambiguous assignment of the aspartic acid spin systems is of

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Fig. 1 a, Portion of the 500-MHz ^1H -NMR spectrum of haemagglutinin peptide (YPYDVDPYA) at 278 K. All spectra were recorded using a Bruker AM500 spectrometer. Dioxan (d) was used as an internal standard, but the spectrum is referenced to TSS at 0 parts per 10^6 (p.p.m.). pH values in D_2O solution refer to uncorrected meter readings. Peptide concentration ~ 10 mM in D_2O , adjusted to pH 4.15 with 0.1 M NaOD and 0.1 M DCl solutions in D_2O . The residual HOD peak was suppressed by gated irradiation. Resolution was enhanced by lorentzian-to-gaussian weighting. **b**, Portion of the aliphatic region of a phase-sensitive, double quantum filtered COSY spectrum²⁶ of peptide. The spectrum was obtained at 278 K using the same solution as that used to obtain the spectrum in **a**. The residual HOD resonance was suppressed by gated irradiation for 2 s before acquisition. A total of 512 points were acquired in the t_1 domain, with 16×2 K free induction decays acquired for each t_1 value. The spectrum was Fourier transformed in both dimensions using a phase-shifted sine bell window function (shifted $\pi/4$ in t_1 and $\pi/8$ in t_2). Connectivities for the proline residues (P2 and P6) and the aspartate residues (D4 and D7), and the α - β connectivities for valine (V5) and tyrosine (Y1, Y3 and Y8) are indicated for the *trans* form only. All other cross-peaks arise from resonances of the *cis* form. **Methods.** The peptide was synthesized on a Bioscience Sam Two peptide synthesizer and purified by HPLC as reported previously³⁵. The sequence of the peptide corresponds to residues 98–106 of the HA1 chain of the H3 subtype X:47 (A/Victoria/3/75) haemagglutinin¹⁶. The immunological properties of this peptide have been studied in detail¹⁶. The affinity of the monoclonal anti-peptide antibodies for the peptide is in the range 1.6×10^5 – 3.5×10^6 M^{-1} and for the haemagglutinin monomer 8×10^5 – 4×10^6 M^{-1} (K. Bergmann, T. Klinger and I.A.W., unpublished observations).



particular importance. Assignments were therefore made by isotopic substitution; a peptide was synthesized in which the amide N of Asp 4 was substituted with $>90\%$ ^{15}N . This nucleus has spin $1/2$ and causes splitting of the resonance of the attached proton. The spectrum of the ^{15}N -substituted peptide (Fig. 3b) allowed unequivocal assignment of the Asp 4 and Asp 7 proton resonances (both *trans* and *cis* forms).

Several NMR parameters provide information on molecular conformation. The temperature dependence of amide proton resonances can be used to identify backbone hydrogen bonding. Except for Asp 4 (*trans* form), the NH proton resonances show the characteristic temperature dependence expected for random-coil peptides, where amide protons are completely exposed to solvent (Table 2). The temperature coefficients of amide proton resonances are expected to be -6 to -10 parts per 10^9 (p.p.b.) per K for random-coil peptides in H_2O (ref. 28). A value of -3.5 p.p.b. per K for Asp 4 NH indicates that this proton is protected from solvent to a considerable extent; it is also protected from solvent at low pH. Since its temperature coefficients are the same in all conditions studied, the *cis* form may be used in this system as an unfolded control. In the presence of 6 M urea, the temperature coefficient for the Asp 4 (*trans*) NH is close to that for the *cis* form (Table 2). We conclude that the *trans* form is unfolded in the presence of urea. In a small monomeric peptide, protection from solvent is probably due to

Table 1 Assignment of αCH and NH resonances of haemagglutinin peptide

		298 K		278 K	
		<i>trans</i>	<i>cis</i> *	<i>trans</i>	<i>cis</i> *
Tyr 1	αCH	4.44	3.506	4.442	3.493
Pro 2	αCH	4.44		4.441	
Tyr 3	αCH	4.575	4.669	4.534	4.668
	NH	7.788	8.191	7.992	8.365
Asp 4	αCH	4.624	4.60	4.607	
	NH	8.242	8.490	8.320	8.605
Val 5	αCH	4.365	4.383	4.333	4.361
	NH	7.937	7.896	8.117	8.188
Pro 6	αCH	4.284	4.313	4.259	4.291
Asp 7	αCH	4.57	4.57	4.523	
	NH	8.280	8.330	8.449	8.504
Tyr 8	αCH	4.55	4.57	4.560	
	NH	7.941	7.972	8.135	8.155
Ala 9	αCH	4.146	4.15	4.127	
	NH	7.898	7.928	8.038	8.052

Spectra obtained for 10 mM solutions of haemagglutinin peptide in D_2O (αCH resonances) and in 90% $\text{H}_2\text{O}/10\%$ D_2O (NH resonances). The pH of the solution in each case (uncorrected meter readings) was 4.10 ± 0.5 . Dioxan was used as internal standard.

* Due to spectral overlap and low intensity, not all of the resonances from the *cis* form of the peptide could be assigned.

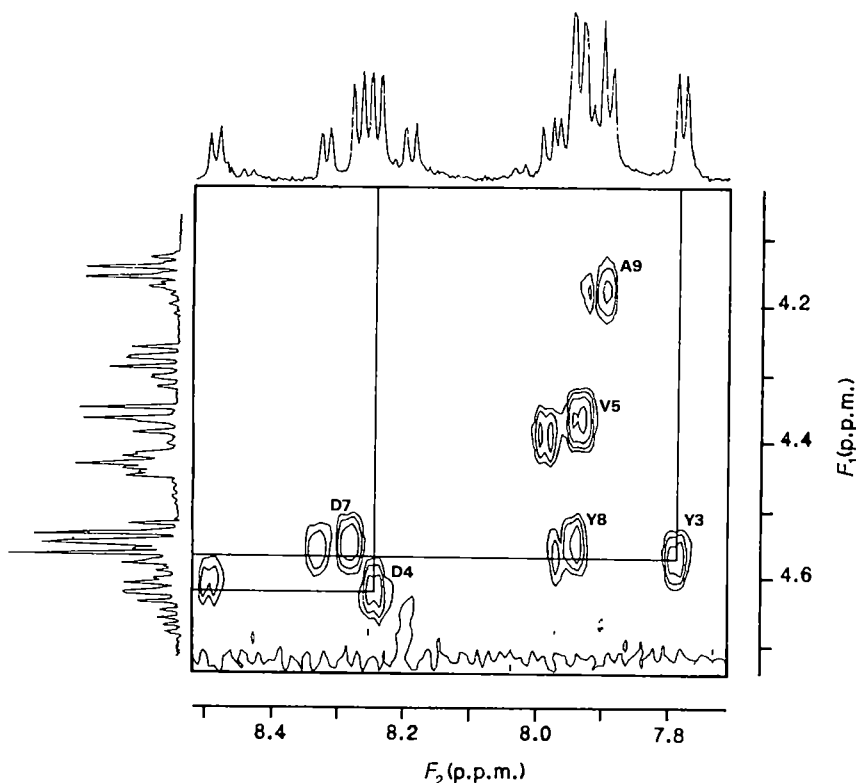


Fig. 2 Portion of the COSY spectrum of haemagglutinin peptide in H_2O solution. The spectrum was obtained using an 8 mM solution of peptide in 5% D_2O /95% H_2O at pH 4.15 and 296 K. A two-dimensional modification of the 'Jump-Return' sequence was used³⁶. 331 time domain points were acquired in t_1 , with 96 free induction decays acquired at each t_1 value. The spectrum was Fourier transformed in both dimensions using a sine bell window function. Cross-peaks between αCH and amide proton resonances are indicated for the *trans* form of the peptide only.

intramolecular hydrogen-bond formation. Reduced temperature coefficients for amide proton resonances have been used frequently to infer the presence of β -turns in peptides^{28,29}. These structures are usually observed in non-aqueous solvents such as dimethyl sulphoxide. In the present case, the temperature dependence of the Asp 4 (*trans*) amide proton resonance suggests participation in an intramolecular hydrogen bond in water solution. We note that the chemical shifts of the amide proton resonances are independent of peptide concentration over the range 1–10 mM, so that interactions between peptide molecules can be effectively discounted.

The existence of a β -bend in the haemagglutinin nonapeptide in water solution is confirmed by measurements of the NOEs. In a reverse turn formed by residues Tyr 1, Pro 2, Tyr 3 and Asp 4, there would be a hydrogen bond between the carbonyl group of Tyr 1 and the Asp 4 NH. Type-I, -II and -III β -turns are possible. In each case a small NOE is expected between the amide proton resonances of residues ($i+2$)(Tyr 3) and ($i+3$)(Asp 4)³⁰. A 2% NOE on the Tyr 3 NH is observed when the Asp 4 NH is irradiated. An NOE of similar size is seen on Asp 4 NH when Tyr 3 NH is irradiated (Fig. 3c). Type-II turns can be distinguished from type-I and -III turns by the presence of a strong NOE between the resonance of the αCH proton of residue ($i+1$) and the resonance of the amide proton of residue ($i+2$)³⁰. In the present case, a small NOE was observed on the αCH proton resonance of Pro 2 on irradiation of the Tyr 3 NH resonance. This NOE could not be readily quantitated because of the proximity of the Pro-2 αCH resonance to the large H_2O peak. In the reverse experiment, in which the αCH resonance of the proline was irradiated, an unequivocal NOE (9%) on the Tyr 3 NH resonance was observed (Fig. 3d). We conclude that, in water solutions of the peptide, a substantial population of conformers contains a type-II turn between residues 1 and 4. The β -turn is found only in the *trans* isomer; there is no evidence for any reverse-turn formation in the minor *cis* isomeric form.

The statistical weights of the three major side-chain rotamers³¹ of each amino-acid residue in the *trans* form of the haemagglutinin nonapeptide were calculated using values of the $^3J_{\alpha H-\beta H}$ coupling constants obtained from computer simulations of the strongly coupled $C\alpha H-C\beta H_2$ fragments of the spin systems. The rotamer populations for Tyr 1 are of particular note: it

appears that the *gauche* rotamer g^+g^- is not significantly populated in the *trans* form of the peptide. The absence of the g^+g^- rotamer is entirely consistent with the presence of a high proportion of molecules containing the β -turn structure, since this conformation would force energetically unfavourable steric crowding between the atoms involved in the turn and the aromatic ring of Tyr 1. We therefore conclude that the β -turn occurs in a high proportion of the conformational states of the *trans* form of the peptide in water solution.

The stable reverse turn reported here is in a peptide which is the site selected by the immune system as the immunodominant region of a larger peptide antigen. This observation bears significantly on several fundamental issues, including the problem of protein folding. Stable local structures, which may be different from those in the final folded protein, can be recognized by antibodies, and have been postulated as initiation sites in folding^{18,19,22,23}. Also, on theoretical grounds, reverse turns have been suggested as intermediates in protein folding²⁰. The experimental observation described here may thus link the immunological and theoretical approaches to protein folding.

Table 2 Temperature coefficients (p.p.b. per K) for NH resonances of haemagglutinin peptide

	pH 4.18		pH 1.50		pH 5.88 +6 M urea	
	<i>trans</i>	<i>cis</i>	<i>trans</i>	<i>cis</i>	<i>trans</i>	<i>cis</i>
Tyr	—	—	—	—	—	—
Pro	—	—	—	—	—	—
Tyr	-9.7	-8.0	-9.1	-7.3	-10.2	-7.6
Asp	-3.5	-5.7	-4.3	-5.8	-5.6	-6.3
Val	-8.3	-9.3	-7.3	-9.0	-9.0	-9.3
Pro	—	—	—	—	—	—
Asp	-8.3	-8.6	-8.1	-8.4	-8.7	-8.9
Tyr	-9.0	-9.1	-9.8	-9.0	-8.3	-8.3
Ala	-6.7	-6.7	-6.8	-6.8	-8.0	-7.9

Temperature coefficients were calculated by a linear least-squares fit to up to 12 data points for each resonance. The temperature coefficients recorded at all pH values above 4 were very similar, as expected since there are no deprotonations in the molecule between pH 4 and neutral pH. This suggests that the structure probably persists at neutral pH.

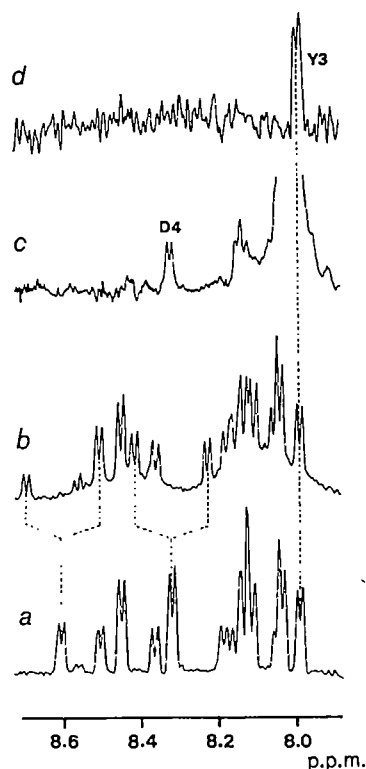


Fig. 3 *a*, Amide proton region of ^1H -NMR spectrum of the haemagglutinin peptide (10 mM in 90% $\text{H}_2\text{O}/10\%$ D_2O , adjusted to pH 4.10 with 0.1 M NaOH and 0.1 M HCl). The spectrum was obtained at 278 K using the Jump-Return sequence³⁷. Resolution was enhanced by lorentzian-gaussian transformation. 2,400 transients were collected. *b*, Amide proton region of spectrum of the haemagglutinin peptide with Asp 4 >90% enriched with ^{15}N . The spectrum shows splitting of the Asp 4 (*cis*) and Asp 4 (*trans*) NH resonances due to $^1J_{\text{NH}}$ coupling. The ^{15}N - β -benzyl-L-aspartate and ^{15}N -*tert*-butoxycarbonyl- β -benzyl aspartate intermediates for the synthesis of this peptide were prepared from >90% L-aspartic- ^{15}N acid (MSD Isotopes) by standard procedures^{38,39}. Peptide concentration was ~2 mM in 90% $\text{H}_2\text{O}/10\%$ D_2O at pH 4.07 at 278 K. *c*, NOE difference spectrum with 1.5-s gated irradiation of Tyr 3 NH resonance at 7.99 p.p.m. Total numbers of transients accumulated, 1,234. The free induction decay was subjected to exponential multiplication before Fourier transformation. *d*, NOE difference spectrum with 1.5-s gated irradiation of Pro-2 αCH resonance at 4.44 p.p.m. Total number of transients accumulated, 952. Resolution was enhanced by lorentzian-gaussian transformation. For the NOE experiments, spectra with the decoupler on- and off-resonance were interleaved (8 transients of each) and stored on the disk.

The main immunological question raised by this work is whether the presence of a stable reverse turn in a synthetic immunogen in water is important for recognition of the cognate sequence in the folded protein. A related question involves the extent to which antibody binding influences the conformation of the protein and the peptide. Previous studies using anti-protein antibodies have suggested that interaction with the antibody may itself introduce order into a peptide^{22,23,32-34}. In the present situation, the conformation of the peptide in solution and the cognate site in the crystal structure of the protein are different, suggesting that one or both must be altered in the antibody-binding site. Since we are dealing with a monoclonal antibody, the most direct approach to answering such questions is to determine the conformation of the synthetic immunogen when bound to the antibody. These studies are in progress, using both crystallographic and NMR methodologies.

Pending results from these studies, we can state our current view of how the immunological issues can be resolved. It seems that there may be a complementarity between the view that the site in the protein to which antibody binds is mobile and the postulate that the immunogenic peptide may be partially

ordered. In keeping with the diversity of the immune system, we would not expect all anti-peptide antibodies to be the same. Sometimes the balance will be towards disorder in the protein whereas at other times more order in the immunogen will be required.

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Note added in proof: NMR studies of a peptide corresponding to an antigenic region of myelin basic protein have now been reported⁴⁰.

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Identification of kinesin in sea urchin eggs, and evidence for its localization in the mitotic spindle

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To understand the molecular basis of microtubule-associated motility during mitosis^{1,2}, the mechanochemical factors that generate the relevant motile force must be identified. Myosin, the ATPase that interacts with actin to produce the force for muscle contraction and other forms of cell motility³, is believed to be involved in cytokinesis but not in mitosis⁴⁻⁷. Dynein, the mechanochemical enzyme that drives microtubule sliding in eukaryotic cilia and flagella^{8,9}, has been identified in the cytoplasm of sea urchin eggs¹⁰⁻¹⁹, but the evidence that it is involved in

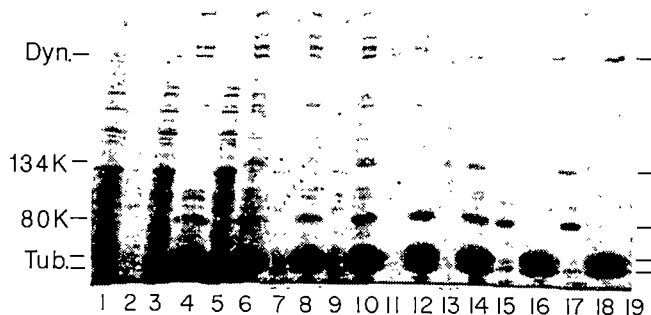


Fig. 1 Specific AMPPNP-dependent co-pelleting of the 134K polypeptide with unfertilized egg cytoplasmic microtubules. The figure shows gels of the supernatants (lanes 1, 3, 5) and pellets (lanes 2, 4, 6) obtained from different experiments. Lanes 1, 2, plus AMPPNP plus colchicine (to block microtubule assembly); lanes 3, 4, no additions; lanes 5, 6, plus AMPPNP. Note that the 134K protein is only recovered in pellet 6, containing AMPPNP but not colchicine. Control microtubule (lane 4) and 'AMPPNP' microtubule (lane 6) pellets were washed to yield the supernatants and pellets shown in lanes 7, 8 and 9, 10, respectively. These pellets were subsequently extracted with 5 mM Mg-ATP, yielding supernatants and pellets shown in lanes 11 and 12, and 13 and 14, respectively. Note that the putative cytoplasmic dynein heavy chain (Dyn.) and the 134K polypeptide remain bound to the AMPPNP-microtubules through the washing step, but are both partially extracted by ATP (lane 13). The rest of the MAPs were extracted from the control and AMPPNP microtubules by differential centrifugation in 0.5 M NaCl plus 0.1 mM ATP; the corresponding supernatant and pellets are shown in lanes 15 and 16 and 17 and 18, respectively. Lane 19 is sea urchin sperm flagella 21S dynein. Tub., tubulin; 80K, an 80,000- M_r MAP.

Methods. Unfertilized sea urchin eggs were washed, dejellied and homogenized in 'extraction buffer' (0.1 M PIPES, 2.5 mM $Mg(CH_3COO)_2$, 5 mM EGTA, 0.1 mM EDTA, 0.9 M glycerol, 0.1 mM phenylmethylsulphonyl fluoride, $1 \mu g ml^{-1}$ pepstatin, $1 \mu g ml^{-1}$ leupeptin, $10 \mu g ml^{-1}$ aprotinin, 0.5 mM dithiothreitol, pH 6.9), then centrifuged to yield the cytoplasmic extract¹³ in which microtubules were assembled by incubation with taxol and GTP^{13,28} for 20 min. 0.1 M AMPPNP in extraction buffer was then added to a final concentration of 5 mM to one aliquot for a further 10 min, while a control aliquot was incubated with an equal volume of extraction buffer. One sample of extract was treated with 100 μM colchicine instead of taxol to inhibit microtubule assembly, then supplemented with 5 mM AMPPNP. These extracts were centrifuged through a 15% sucrose cushion¹³. The SDS gel system used here contained 6% acrylamide plus 0.06% bis-acrylamide. The low concentration of cross-linker allows easy visualization of the dynein heavy chain. Other biochemical procedures were performed as described previously¹³.

cytoplasmic microtubule-based motility (rather than serving as a precursor for embryonic cilia) is equivocal. Microtubule-associated ATPases have been prepared from other tissues (reviewed in ref. 12), but their role in cytoplasmic motility is also unknown. Recent work on axoplasmic transport, however, has led to the identification of a novel mechanochemical protein called kinesin²⁰, which is thought to generate the force for moving vesicles along axonal microtubules²⁰⁻²⁷. These results suggest that kinesin may also be a mechanochemical factor for non-axoplasmic forms of microtubule-based motility, such as mitosis. We describe here the identification and isolation of a kinesin-like protein from the cytoplasm of sea urchin eggs. We present evidence that this protein is localized in the mitotic spindle, and propose that it may be a mechanochemical factor for some form of motility associated with the mitotic spindle.

Extracts of squid axoplasm will support a microtubule-based, ATP-dependent movement of vesicles²⁰⁻²⁷. This motility is driven by a translocator that exists primarily in soluble form, with a fraction serving to cross-link microtubules to the moving vesicles²⁴. The non-hydrolysable ATP analogue, adenylyl imidodiphosphate (AMPPNP) 'freezes' vesicle motion on microtubules^{22,24,26}, suggesting that the analogue induces strong bind-

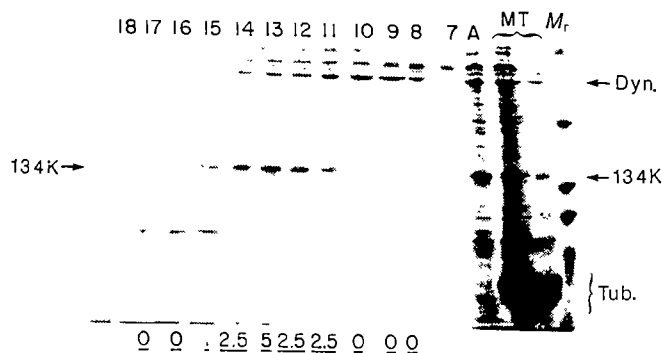


Fig. 2 Biogel A5M chromatography of sea urchin egg MAPs. Microtubules (MT, two different loadings) prepared in AMPPNP were washed in extraction buffer supplemented with 75 mM NaCl. The 134K polypeptide (134K) and putative dynein heavy chain (Dyn.) were extracted from the microtubules by differential centrifugation in buffer containing 0.1 M NaCl/10 mM $MgSO_4$ /7.5 mM ATP (lane A), then fractionated on a 1.2×20 -cm column of Biogel A5M. Fractions of 50 drops were collected, then 40- μl aliquots were analysed on SDS gels (lanes 7-18). Fraction 8 represents the void volume (V_0) and fraction 17 is the included volume (V_i). Aliquots (100 μl) of each fraction were frozen in liquid nitrogen, then transported on dry ice by Federal Express to the Marine Biological Laboratories, Woods Hole, where R. D. Vale and M. P. Sheetz analysed them for their ability to induce microtubules to move relative to other microtubules 'in vitro' as described by Vale *et al.*²⁰ for squid and bovine brain kinesin. The numbers beneath each column fraction represent the maximum dilution factor at which motility-inducing activity can still be detected. 0 = No motility observed. Vale and Sheetz did not know the polypeptide composition of the samples before assay. Note that motility-inducing activity co-elutes with the 134K polypeptide, the most active fraction being 13 ($K_{av} = 0.56$).

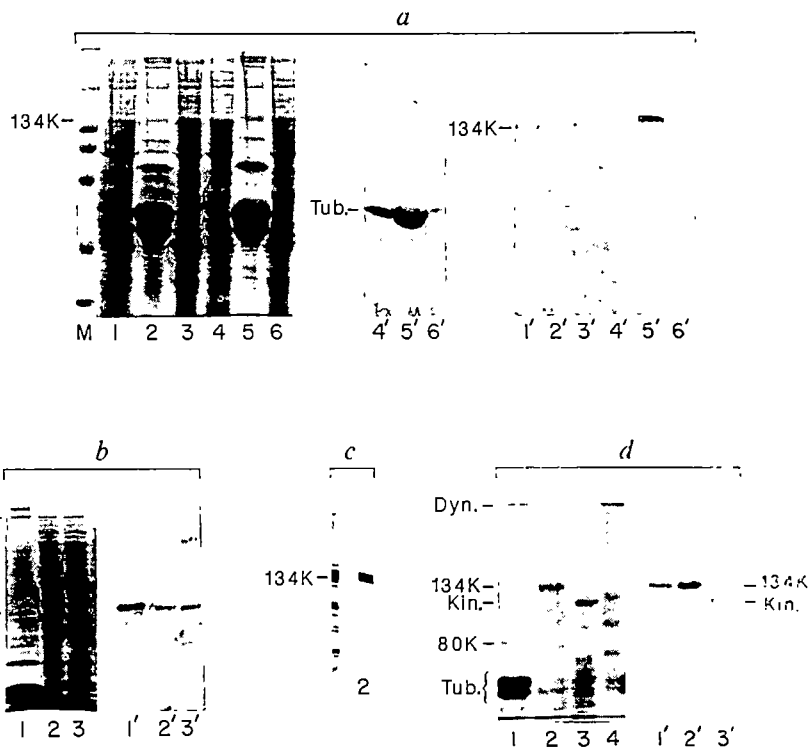
ing between the translocators and microtubules, thereby driving the translocators from a soluble to a bound form. We reasoned that AMPPNP might enhance microtubule binding of the translocators present in cytoplasmic extracts, so that microtubules prepared in AMPPNP might serve as an affinity matrix for identifying and isolating these mechanochemical proteins.

Taxol-assembled microtubules were therefore prepared from cytoplasmic extracts of unfertilized sea urchin eggs^{13,28} in the presence and absence of 1-5 mM AMPPNP. The amount of various microtubule-associated proteins (MAPs) co-sedimenting with tubulin was determined by densitometry of SDS-polyacrylamide gels (Fig. 1). We previously reported that a cytoplasmic dynein-like protein present in sea urchin egg extracts exists in both soluble and microtubule-associated forms^{13,14}. Only a small increase in the amount of the putative dynein heavy chain was observed in microtubules pelleted in the presence compared with the absence of AMPPNP (0.6 compared with 0.4 mol per 100 mol tubulin). Furthermore, we observed no significant increase in the ATPase activity which co-pelleted with the microtubules in the presence of AMPPNP (microtubules + AMPPNP: specific activity ~ 10 nmol per min per mg, total activity 31 nmol per min; microtubules - AMPPNP: specific activity ~ 13 nmol per min per mg, total activity 36 nmol per min). These results suggest that cytoplasmic dynein interacts with microtubules differently from the squid axoplasmic vesicle translocator^{22,24,26}; they do not support the hypothesis that cytoplasmic dynein is the motor for vesicle transport.

Addition of AMPPNP did, however, greatly enhance the amount of a polypeptide of relative molecular mass (M_r) 134,000 (134K) which co-sedimented with microtubules (Fig. 1) (~ 8 mol 134K per 100 mol tubulin compared with ~ 1 mol 134K per 100 mol tubulin without AMPPNP). Identical results were obtained when an egg extract was treated with 1 mM ADP plus 100 μM Na_3VO_4 , or when the ATP concentration in the extract was depleted by incubation with apyrase. The 134K polypeptide

Fig. 3 Antibody to the sea urchin egg 134K polypeptide stains a 134K polypeptide in immunoblots of cytoplasmic microtubules prepared in AMPPNP (*a*) and isolated mitotic spindles (*b*). It also reacts with the 110K polypeptide of squid kinesin (*d*). *a*, 7.5% SDS gels of microtubules prepared in the presence and absence of AMPPNP (Fig. 1). Lanes 1–6, Coomassie blue-stained gel; lane M shows M_r markers: 200,000; 116,000; 96,000; 68,000; 44,000; and 29,000. Lanes 1, 4, cytoplasmic extracts; lane 2, microtubules prepared in the absence of AMPPNP; lane 5, microtubules prepared in the presence of AMPPNP; lanes 3, 6, the corresponding supernatants. Immunoblots of corresponding lanes (1'–6') probed with anti-tubulin¹³ (Tub.) and 134K antibody are also shown. The 134K antibody reacts with a 134K polypeptide in microtubules pelleted in the presence (lane 5') but not in the absence (lane 2') of AMPPNP. *b*, The 6% polyacrylamide gels (lanes 1–3) and corresponding anti-134K immunoblots (lanes 1'–3') show microtubules prepared in the presence of AMPPNP (lanes 1, 1') and mitotic spindles which were isolated³² in the presence (lanes 2, 2') and absence (lanes 3, 3') of AMPPNP. An immunoreactive 134K polypeptide is present in spindles isolated either way. *c*, Blot-affinity purification^{29–31} of 134K antibodies. Lane 1, a nitrocellulose strip of 'AMPPNP' microtubules probed with whole 134K antiserum. Lane 2 was probed with affinity-purified antibodies eluted from 134K polypeptide/nitrocellulose strips²⁹. *d*, Cross-reaction of sea urchin egg 134K antibody with squid axoplasmic kinesin. The Coomassie blue-stained gel (lanes 1–4) and corresponding anti-134K immunoblot (lanes 1'–3') show microtubules prepared from fertilized sea urchin eggs in the presence of AMPPNP (lanes 1, 1'), sea urchin egg kinesin (lanes 2, 2') and purified squid axoplasmic kinesin (lanes 3, 3'). Lane 4, a 21S dynein marker. The 134K urchin polypeptide and the 110K squid kinesin polypeptide (Kin.) are indicated.

Methods. Two rabbits were injected at 2–3-week intervals with 134K polypeptide excised from stained-destained SDS gels of proteins extracted from 'AMPPNP-microtubules' in ATP + NaCl, over a period of 4 months. No 134K antibodies were detected in preimmune sera from either rabbit, but 134K antibodies could be detected on immunoblots at dilution 1:1,000 after the first and all subsequent booster injections.



did not pellet from AMPPNP-treated cytoplasmic extracts of unfertilized sea urchin eggs in which microtubule assembly was inhibited by treatment with colchicine (Fig. 1). Therefore, in the presence of AMPPNP, the recovery of this polypeptide in the microtubule pellet depends on its binding to the microtubules. The polypeptide composition of taxol-assembled microtubules prepared in the presence of AMPPNP was unchanged by the addition of 0.1% Triton X-100, suggesting that the 134K polypeptide is not associated with membranous vesicles. Similar results were obtained when cytoplasmic extracts of fertilized eggs were used to prepare microtubules.

Like the cytoplasmic dynein heavy chain, the 134K polypeptide was not released from the microtubules by differential centrifugation in the original egg extraction buffer containing 75 mM NaCl, but it was partially extracted using 5–10 mM Mg-ATP, and completely extracted using 0.5 M NaCl or 0.1 M NaCl, 7.5–10 mM ATP plus 10 mM MgSO₄ (Figs 1, 2). As the latter procedure gave the most efficient and specific extraction of 134K protein, this material was used for further purification by gel filtration chromatography (Fig. 2).

The sea urchin egg 134K protein resembles kinesin²⁰ in several respects. (1) The binding of both proteins to microtubules is greatly enhanced by AMPPNP; (2) they are extracted from microtubules in 0.1 M NaCl plus 10 mM Mg-ATP; (3) their elution behaviour on gel filtration columns suggests that they contain multiple subunits; (4) fractions containing the sea urchin egg 134K polypeptide cause microtubules to move relative to other microtubules, and beads to translocate along microtubules 'in vitro' in a manner very similar to squid kinesin (Fig. 2). These results strongly suggest that the 134K polypeptide represents sea urchin egg kinesin.

Specific antisera were raised against the sea urchin 134K polypeptide by injecting rabbits with electrophoretically purified 134K polypeptide. These antibodies stained the 134K polypep-

tide present in immunoblots of microtubules pelleted from egg extracts in the presence of AMPPNP (Fig. 3). This 134K antigen was enriched in microtubule pellets relative to the original cytoplasmic extracts, but it was not observed in immunoblots of pellets prepared in the absence of AMPPNP (Fig. 3). To analyse the immunological relatedness of the sea urchin 134K polypeptide and squid axoplasmic kinesin, blots of both proteins were probed with the anti-134K antiserum (Fig. 3d) or with blot-affinity-purified 134K antibody (Fig. 3c)^{29,31}. The 134K antibody exhibited a clear cross-reaction with the major polypeptide component (M_r ~110K) of purified squid axoplasmic kinesin. Furthermore, antibodies affinity-purified from the 134K antisera on blots of the squid kinesin 110K polypeptide also cross-reacted with the sea urchin 134K polypeptide (data not shown). These data strongly suggest that the sea urchin egg 134K polypeptide represents kinesin. It is interesting that the M_r s of squid axoplasmic kinesin (110,000; ref. 20) and sea urchin egg kinesin (~134,000) are clearly different (Fig. 3).

The observation that a kinesin-like protein is associated with microtubules from the cytoplasm of sea urchin eggs, where chromosome movement is a major function of these structures, suggested that kinesin might be a component of the mitotic spindle. Immunoblots of mitotic spindles isolated from sea urchin embryos³² showed that antibodies to the 134K protein recognized a polypeptide of appropriate M_r in spindles isolated with or without AMPPNP. It is uncertain why AMPPNP is not required for association of the 134K protein with spindle isolates. However, since the isolation protocol involves egg lysis in ~100 volumes of buffer containing no ATP, the resulting depletion of ATP concentration may be sufficient to enhance microtubule-binding by the 134K protein.

The localization of the 134K polypeptide in fixed mitotic sea urchin eggs was investigated by immunoperoxidase light microscopy using blot-affinity-purified 134K antibodies (Fig. 4)

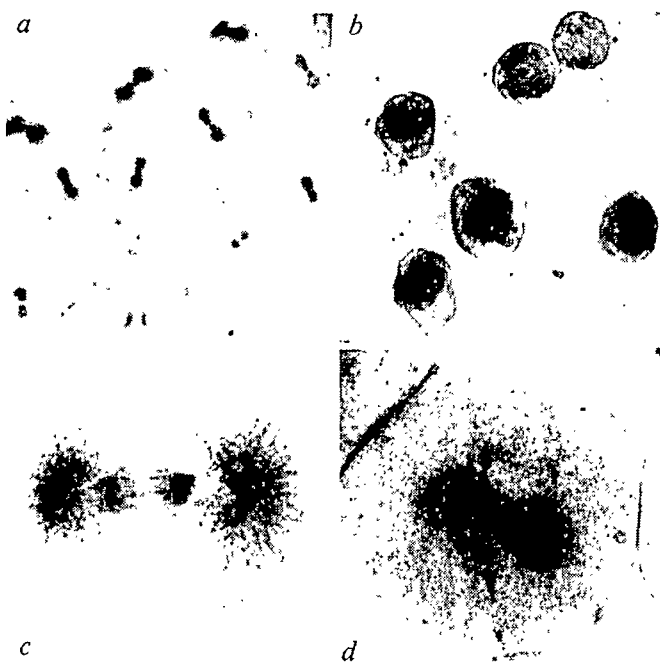


Fig. 4 Immunoperoxidase light microscopic localization of the 134K kinesin-like protein in dividing sea urchin embryos. *a*, A low-magnification view of lysed cells stained with blot affinity-purified 134K antibody, showing clear spindle staining. Whole immune sera (diluted 1:100–1:1,000) from both rabbits also exhibited clear spindle staining, whereas corresponding preimmune sera exhibited no microtubule staining. *b*, Control lacking primary antibody. *c*, High-magnification view of a lysed cell stained with blot-affinity-purified 134K antibody, showing clear localization of the 134K polypeptide in the spindle fibres. *d*, Staining of an unlysed dividing blastomere. Note the concentration of the 134K protein in the mitotic spindle region. The approximate magnifications are: *a*, *b*, $\times 100$; *c*, $\times 700$; *d*, $\times 500$.

Methods. Sea urchin eggs were fertilized, allowed to progress to metaphase and fixed in 90% methanol plus 50 mM EGTA with or without prior lysis in 0.5% Nonidet P-40 (ref. 37). The fixed cells were incubated with blot-affinity-purified 134K antibodies and processed for peroxidase-antiperoxidase (PAP) staining and bright-field light microscopy.

The antibodies were checked for monospecificity on immunoblots of whole egg homogenates before use. Spindle staining was obvious (Fig. 4*a*). Observations of unlysed metaphase eggs stained with the affinity-purified 134K antibody revealed that the corresponding antigen is present throughout the cytoplasm, but is highly concentrated in the mitotic spindle (Fig. 4*d*). This pattern may reflect the existence of pools of soluble kinesin (accounting for background staining) and microtubule-associated kinesin (producing the spindle staining). Tubulin is believed to exhibit a similar distribution in dividing sea urchin eggs³³. When the blastomeres were lysed with detergent before fixation, clear staining of both chromosome-to-pole and astral fibres was evident (Fig. 4*c*). Several control experiments confirmed that staining was specific for 134K antibodies. (1) No microtubule staining was observed with preimmune sera or when the cells were incubated with secondary antibodies alone (Fig. 4*b*). (2) When 134K antibodies were preincubated with the 134K protein prepared by gel filtration chromatography (Fig. 2), no microtubule staining was observed by subsequent immunoperoxidase light microscopy. (3) Preabsorption of the 134K antibody with bovine brain tubulin did not affect staining. (4) Antibodies prepared from the 134K antisera by blot-affinity purification against the squid kinesin 110K polypeptide also stained the spindle fibres by immunoperoxidase light microscopy. These results strongly suggest that spindle staining is not due to a contaminating 134K polypeptide in our sea urchin microtubule preparations, but rather to the presence of a kinesin-like protein in the spindle fibres.

We propose that the 134K polypeptide represents a cytoplasmic form of kinesin which is associated with the mitotic spindle in dividing sea urchin embryos. The observations that kinesin induces microtubule-based motility *in vitro* suggest that it may also promote motility within the cell, so it is tempting to speculate that the sea urchin protein performs a mechanochemical function within the mitotic spindle. For example, it may cause microtubules to slide relative to other microtubules or to some other components of the spindle, or it may cause chromosomes to be translocated along microtubules. Alternatively, cytoplasmic kinesin may not be directly involved in chromosome movement, but may instead be involved in moving 'particles or states' along microtubules in the metaphase half-spindle (see, for example, ref. 34). To distinguish between such possibilities and to elucidate the function of kinesin within the cell, other techniques such as antibody microinjection into living cells³⁵ are being used.

A predicted property of kinesin is ATPase activity. Interestingly, since this manuscript was submitted, Brady reported³⁶ that the AMPPNP-dependent binding of a 130K polypeptide to chick brain microtubules accompanies an increase in the ATPase activity of the microtubules. However, the exact relationship between this ATPase and kinesin is not yet established.

We thank Drs Ron Vale and Mike Sheetz of the NIH NINCDS group at the Marine Biological Laboratories, Woods Hole, for providing squid axoplasmic kinesin and for performing motility assays on the sea urchin egg protein. Their results and the characterization of the sea urchin protein will be described elsewhere. We also thank numerous colleagues for their interest in this work, Larry Harwood for photography and Cathy Inouye and Karen Brown for secretarial assistance. J.M.S. particularly thanks Bonnie Neighbors for advice on immunoperoxidase light microscopy. Taxol was a gift of Dr M. Suffness (National Products Branch, NCI). J.M.S. was supported by an MRC Travelling Fellowship and a British-American exchange fellowship from the British Heart Foundation and American Heart Association. M.E.P. was supported by a fellowship from the Helen Hay Whitney Foundation. This work was supported by NIH grant GM 30213 and American Cancer Society grant BC-498 to J.R.M.

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A molecular solution to the riddle of the giant panda's phylogeny

O'BRIEN *et al.*¹ predict "the existence of older Pliocene or even Miocene fossils of the ancestors of the giant panda". In fact, a putative ancestor was identified by Thenius² in 1979. This is *Agriarctos Kretzoi*, described on mandibular dental material from the late Miocene of Hungary. This ursid shows incipient molarization of the premolars apparently foreshadowing the condition in *Ailuropoda*.

This is independent support of the conclusions reached by O'Brien *et al.*, including their suggestion that *Ailuropoda* (with *Agriarctos*) may be assigned to a subfamily (*Ailuropodinae*) of the family Ursidae.

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Steens Mountain geomagnetic polarity transition is a single phenomenon

IN their reinterpretation of the transition recorded in the Steens Mountain lavas¹, Valet *et al.*² suggest that the second phase (the 'rebound') occurred at a significantly later time than the first phase (the reverse-to-normal transition). In this way they attempt to separate what is one phenomenon into two separate phenomena, and assert that the second phase is a geomagnetic excursion unrelated to the preceding reversal. Several lines of evidence indicate that there was no significant hiatus in eruptive activity in the part of the lava section on Steens Mountain sampled by us and previously by Watkins³.

First, there are no soil horizons, weathered zones or interbasaltic sedimentary rocks or sediments anywhere in the sampled section, except some negligible baked earths (tuffs, perhaps) that we found in two restricted localities stratigraphically unrelated to the limits of either of the two transition phases. Moreover, the lavas are of rather uniform composition, and the only obvious lithological variability between successive lavas is the presence or absence of large platy labradorite phenocrysts up to 4 cm in diameter. The occurrence of these porphyritic lavas is unrelated to any aspect of the polarity transition.

Second, Gunn and Watkins^{4,5} made many complete chemical analyses of the

Table 1 Chemical groups⁵ and directional groups¹ for Steens lava analyses

Chemical group	Directional group
A, B	1-10
C	10-12
D	12-20
E	21-26
F	26-28
G	30-42
H	42-55

Table 2 Recalculated K-Ar ages

Watkins flow no. ^{1,5}	Directional group ¹	Age (Myr)
11	~4	15.5 ± 0.15
17	~10	15.4 ± 0.13
51	33	15.5 ± 0.28
61	43	15.4 ± 0.25
68	49	15.6 ± 0.21
70	49	15.3 ± 0.21

The uncertainty is 1 s.d. of the data.

Steens lavas. Table 1 shows how their chemical groups correspond to our directional groups. The first phase of the reversal begins with directional groups 43 and ends with group 30, and the second phase begins with group 28 and ends with group 15. The variations in chemical composition are uncorrelated with any aspect of the palaeomagnetic record, and several of the chemical boundaries occur within directional groups. The only possible peculiarity is that Gunn and Watkins analysed a high-alumina flow and a pyroxene-rich flow in chemical group F, which corresponds to the beginning of the second phase. These lavas, however, are perfectly correlated with the others, as demonstrated by an alumina variation diagram⁵.

Third, there is no discernible age difference between the top and bottom of the sampled section. We have recalculated

and averaged the K-Ar ages published by Baksi *et al.*⁶ and they are given in Table 2 (new decay constants).

Thus, the available field observations and geochemical and geochronological data directly contradict the suggestion of Valet *et al.*² that the two phases described in our article¹ may not belong to a single geomagnetic process.

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VALET, LAJ AND TUCHOLKA REPLY—

In some of the reversal records that we have obtained from sedimentary sections in Greece (work partly published and presented at IAGA Praga 1985), we have also observed excursions occurring either before or after the transition. In all these cases the characteristics of the directional changes during both the polarity transition and the excursions are similar. The continuity of the sedimentary records also shows that the duration of the excursions never exceeds the time span of the transition.

In the Steens Mountain record¹, the first phase is quite similar to a sedimentary reversal record but, in striking contrast to our observations, the second phase is dissimilar. Furthermore, between the two phases the geomagnetic field has recovered high intensity and dipolar direction. The authors consider the two phases as a single phenomenon. In an attempt to reconcile sedimentary and volcanic records, we have suggested² that the widely different symmetry properties could indicate two unrelated different phenomena.

The existence of rebounds or oscillations associated with a reversal is an important problem which, as Prévot *et al.* suggest¹, may be crucial to our understanding of geomagnetic reversals. Clearly, what is needed is a criterion to establish when an excursion is connected to a reversal. The argument of continuity

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suggested by Prévot *et al.* can reasonably be used as such a criterion. However, if the continuity can usually be well established in sedimentary records, this appears to us much more difficult in volcanic records. Furthermore, despite the additional information given here by Grommé *et al.*, we are not fully convinced that any of their arguments provide compelling evidence of a continuous mean extrusion rate on a timescale of less than 1,000 yr.

The rather uniform composition of lavas can hardly be used in this sense, as periods of constant chemical composition lasting up to about 150,000 yr have been observed, for example, in Etna³. We agree that the K-Ar dating results really indicate the same age for the top and bottom parts of the sequence. However, differences of about 0.3 Myr inside the same directional group (No. 49) are observed and if standard deviation is taken into account, the time span of age could reach 0.7 Myr.

Finally, the argument of the secular variation used by Prévot *et al.*¹ to establish the linearity of timescale is also not convincing. Neither the number attached to the angular displacement of directions nor the stability of the secular variation over long periods of time is confirmed in longer records of secular variation, in lake sediments⁴ or by comparison of lake sediments and recent lavas⁵.

We are convinced that the data obtained by Prévot *et al.* provide a very impressive description of the geomagnetic field during a transition. What we are saying is that, in the present state of knowledge (or ignorance), part of the interpretation of this record might still be open to discussion.

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Dust production in the Sahel

I WISH to make three points concerning the adequacy and consistency of the Sudanese visibility data used by Middleton in a recent analysis¹ relating the frequency of severe atmospheric dust occurrences to annual rainfall.

First, after 1974, the Sudan Meteorological Service started recording visibility

at 00.00 GMT in addition to 06.00, 12.00 and 18.00. Frequencies of poor visibility presented by Middleton for El Fasher in Fig. 1 (and for five Sudanese stations in Table 2) are therefore inflated for the years 1975-78 compared with previous years. Second, each observation of poor visibility does not necessarily refer to a separate dust storm event. Poor visibility at 06.00, 12.00 and 18.00 may be the continuation of a single storm whereas Middleton would categorize these as three separate storms. The resolution of the Sudanese data is insufficient to make this distinction. Again, the caption for El Fasher in Fig. 1 is misleading and the data are almost certainly inflated.

Finally, and most important, no discussion of the reliability of the visibility readings is included. Atmospheric visibility is a notoriously difficult climatic parameter to quantify. The Sudan Meteorological Service used the system of locating key landmarks at known distances from the observing station which are recorded either as seen or unseen by the observer at the designated times. I have observed ephemeral objects such as trees and electric lights being used at Sudanese stations. I would treat with great caution data collected in such a manner, where differences in procedure between successive observers can be crucial.

An inverse relationship between the recorded frequency of dust events and annual rainfall does appear to exist (subject to my first and second points) and the analysis is useful. I suggest, however, that climatic data from Sahelian countries be treated cautiously and their measurement and collection procedures carefully investigated and understood before they are analysed.

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MIDDLETON REPLIES—I thank Dr Hulme for his interest. Dust storms are recorded by meteorological observers using fixed objects as visibility targets in all countries of the world where such phenomena occur, and such observations do present problems of observer error and involve an element of subjective decision making. As with all meteorological data there may also be changes in observational procedures. There are other problems inherent in interpreting data on the frequency of dust storms and dust storm days; I have highlighted some of the problems in a recent paper¹. Briefly, the statistics may conceal considerable variation in the areal and volumetric extent of dust storms and their duration. However, some of these problems are less serious when a

group of stations is used² to illustrate a particular trend in dust storm activity.

There may well be some inflation in the data for the Sudanese stations since 1974 as a result of a change in the frequency of visibility recording to which Hulme correctly refers, but the increasing trend that started before 1974 has continued to rise since that date and seems to be confirmed by studies in other countries of the Sudano-Sahelian belt. Note also that mean summer concentrations of dust at Barbados were again very high during 1982 and 1983 (ref. 3), although any comparison of dust storm frequency with dust mobilization or transport should be cautious because the scale of dust-raising events and the altitude to which material is lifted are of fundamental importance to the long-range transport of dust, and such information is not provided by dust storm frequency data for a particular station, as mentioned above.

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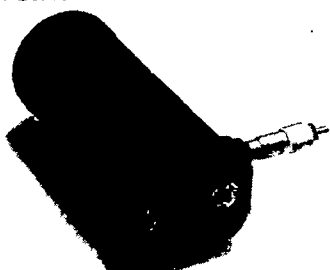
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New looks at spectroscopy

More spectroscopic equipment is becoming interfaceable with desktop computers — and even the Bunsen burner is going high-tech.

- Weighing only 2 pounds and compact in size, the Spectra-Physics IR minimonochromator system (Model SP5155) covers the 2.7–30 μm region. The optical configuration is that of an Ebert monochromator with grating blaze options. Standard interface optics packages are available for interfacing the unit with Spectra-Physics tunable diode lasers and the SP5800 laser source assembly. The minimonochromator is a product of the Laser Analytics Division of Spectra-Physics.

Reader Service No. 100.



IR minimonochromator system SP5155.

- The new RC, high efficiency recirculating coolers from New Brunswick provide a continuous and reliable capacity from 750 to 58,500 watts and allow fluid temperature control between -40°C to $+75^{\circ}\text{C}$ with accuracy of 0.5°C . Coupled with one of the variety of built-in pumps available these recirculating coolers are ideal for the heat removal requirements of most laser systems. The optional digital controller and IEEE/488 bus allow computer control of the system for complete automation.

Reader Service No. 101.

- The Varian VXR series NMR spectrometers are superconducting Fourier transform instruments functioning at 100, 200, 300, 400 and 500 MHz. All critical spectrometer functions are under computer control, and components are modular for high reliability, ease of maintenance and for easy upgrading. For data manipulation and experiment management, the multiple processor-based VXR 4000 data station includes a 68000-based computer and a Winchester capacity of up to 160 Megabytes. The VXR 4000 may use optional networking capabilities including Ethernet, Varian's magNET and Serial Communications software. For data processing and display, the VXR 4000 may be purchased as an independent work station.

Reader Service No. 102.

These notes are based on information provided by the manufacturers. To obtain further details about these products use the reader service card bound inside the journal.

- A self-contained extract system for atomic absorption spectrophotometers is now available in kit form from Mason Nordia. The extract system will protect the operator against toxic fumes and protects the instrument against corrosive vapours. To reduce the risk of heat gain in the laboratory, the kit has specific capability to overcome problems of heat concentration where the flame generates 3kW or more of heat.

Reader Service No. 103.

- The latest catalogue on optical table and isolation systems from Photon Control of Cambridge includes: large high performance metal honeycomb and synthetic granite optical tables designed to meet the needs of the laser and optical scientist; up-to-date modular designed vibration isolation systems; and special purpose tables and vibration isolation systems on which to mount precision instruments and sensitive equipment. Use the readers service to obtain a free catalogue.

Reader Service No. 104.

- Blak-Ray UV intensity meters are designed for monitoring the efficiency and uniformity of UV light sources. The J-225 model measures in the short-wave range from 220 to 280 nm with peak sensitivity at 254 nm. Long-wave readings are made with the J-221 model with a range from 300 to 400 nm and a peak sensitivity at 365 nm. Readings are given in $\mu\text{W cm}^{-2}$. Sensors plug directly into the top of the meter housing. Readings in hard-to-reach locations are made using a 4-foot cable to connect the sensor to the meter. Each model features a A and B Scale for low or high intensity readings. A selector switch chooses the scale, and a snap-on accessory filter provides true UV readings with infrared present.

Reader Service No. 105.

- A new infrared detector is available for the Rofin-Sinar optical spectrum analysis system. The detector enables the system to rapidly scan optical spectra in the 1.5 to 4.5 μm wavelength region with a resolution of 20nm. The resulting spectral data (10 scans per second) may be displayed in real time on a standard laboratory oscilloscope or it may be processed by an Apple or BBC computer before being displayed on a monitor or printer. The detector unit, model RSO 6115, uses a PbSe element as the optical transducer and it is supplied with a stabilizer bias power supply, a signal pre-amplifier, and all the necessary mounting hardware for easy connection to the RSO 6140 scanning monochromator.

Reader Service No. 106.



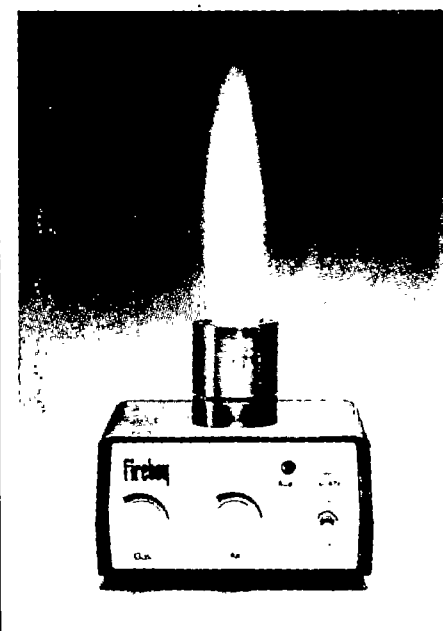
LKB's PC-interfaceable liquid chromatography.

- LKB-Produkter AB of Sweden has developed extended PC software for its diode array detector for use in liquid chromatography. This new Wavescan software makes it possible to automate diode array detection even further, and offers a significant increase in post-run analytical productivity. Designed for the PC, XT, AT or any IBM compatible, Wavescan programs use a simple menu technique. With instant access to isograms, chromatograms and spectra, it is possible to obtain considerably more detailed information on sample identity and purity.

Reader Service No. 107.

High-tech 'Bunsen'

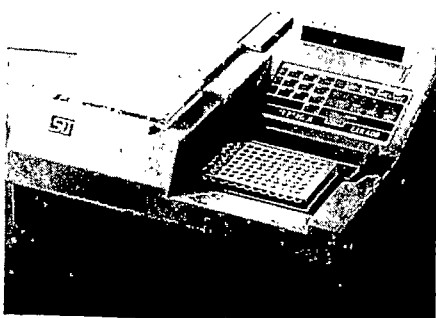
THE conventional Bunsen burner may be a thing of the past. From Technomara of France comes the Fireboy, with an electronically controlled gas flame. Exotic features include a provision for a control pedal operated by the foot to free both hands for experimental procedures, and accurate control of the flame temperature. Reader Service No. 108.



• Questek excimer lasers, available from Lambda Photometrics, provide high power UV pulses (40–750MJ) at any one of a number of fixed wavelengths. A new 20-page brochure is now available, giving full technical details, applications data and suggestions on selecting the right model. *Reader Service No. 109.*

• Australian Monoclonal Development (AMD) now offers a T-cell panel for enumeration of total T cells and T-cell subsets using the indirect immunofluorescence method. The AMD T-cell panel includes three monoclonal antibodies: T4, T8 and T11 equivalents, plus FITC anti-mouse Ig conjugate. Future releases will include a conjugated panel of the same antibodies. These reagents are currently being used routinely to determine T4/T8 ratios in suspected AIDS patients and also to observe T cell imbalances in other immunological diseases. AMD will soon be marketing two other tests for clinical use. The Monohaem kit is a reliable immunoassay for occult blood in faeces and is based on a monoclonal antibody which is specific for human haemoglobin. The AMD Gono-kit uses a panel of monoclonal antibodies which covers all strains of *N. gonorrhoeae* for definitive identification by direct immunofluorescence on clinical specimens. *Reader Service No. 110.*

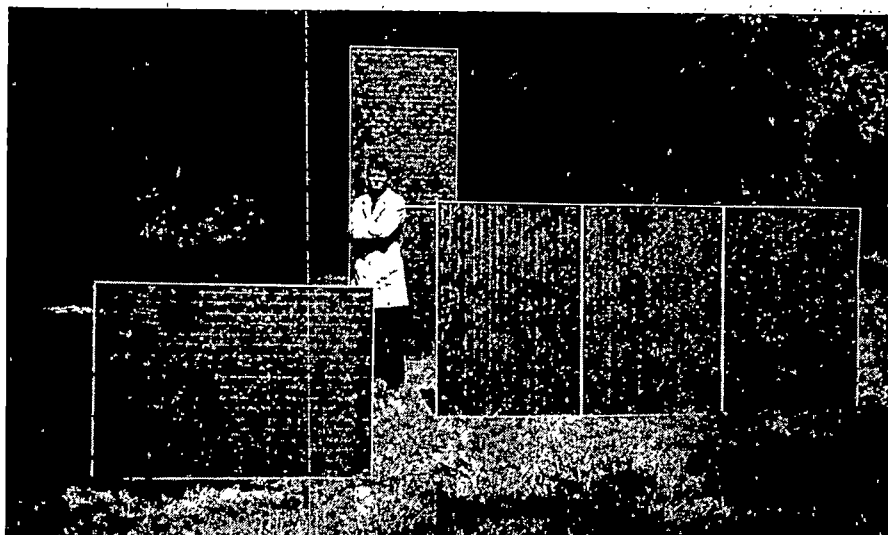
• New from SLT Labinstruments, the SLT Easy Reader plate photometer is designed with enzyme-linked immunosorbent assay in mind. The Easy-Reader is ideal for low volumes, combining fast, accurate measurements with sophisticated software for data evaluation. The Easy



SLT's Easy Reader.

Reader Measures the whole plate with one filter in 20 seconds and for dual wavelength measurements it takes only 35 seconds. *Reader Service No. 111.*

• Hewlett-Packard's HP 9000 computer with enhanced mass-spectrometer software will enhance the performance of the HP 5970B mass-selective detector (MSD), HP 5995C GC/MS, and HP 5988A GC/MS and LC/MS systems. The workstation combines enhanced GC/MS, LC/MS and MSD software with the new HP 9000 Series 300 technical desktop computer, which is designed specifically for instrument control and scientific-data handling. *Reader Service No. 112.*



The largest solar panels on the market, from Solarex. The modules are available as 145, 290 and 435 watt units and achieve a high efficiency of 12.4%. *Reader Service No. 113.*

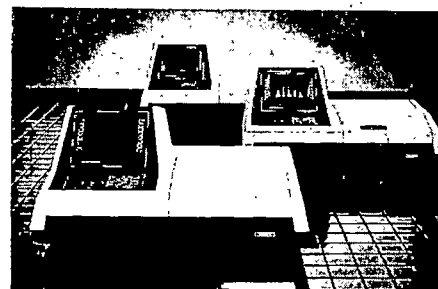
• Now available in the UK from Baird & Tatlock, the Cecil range of UV/visible spectrophotometers provides three single-beam models, each with a choice of four systems. The three models available have a wavelength range of 190 to 990nm and a wavelength accuracy within 1nm. The models are: CE2202, measuring transmittance and absorbance on a meter scale, ideal for teaching or routine work; CE2272, with a linear scale for transmittance, concentration and wide scale expansion ranges for absorbance; and CE2293, with a digital scale for absorbance transmittance and concentration. It also has the option of computer interfacing, data handling and an autozero facility. *Reader Service No. 114.*

• Rigaku manufactures two similar X-ray fluorescent spectrometers — the 3070 and the 3071. The 3070 spectrometer has normal optics; the 3071 features inverse optics. The 3071 allows for greater ease of liquid sample handling and analysis, whilst retaining the same features as the 3070 model. Both are menu-driven for ease of operation, with an internal-external sample chamber for continuous feed analysis, spectrometer status display, full data reduction capabilities, spectrum marker elemental identification and computer controlled pulse height analyser. *Reader Service No. 115.*

• New options on the Perkin-Elmer 1700 Series of Fourier transform infrared spectrometers include an extended near IR range. The standard model 1750 now scans to 5,000 cm^{-1} in the near infrared, a region of the spectrum particularly useful for quantitative analysis in the polymer industry. Model 1710 scans to 4,400 cm^{-1} , and this wavelength range is extended to 5,000 cm^{-1} when the spectrometer is used with the model 3600 data station. And a caesium iodide beamsplitter is now available as an alternative to the standard KBr beamsplitter. Fitted with this, the model 1710 scans from 4,300 cm^{-1} to 220 cm^{-1} and model 1750 from 5,000 cm^{-1} to 220 cm^{-1} . *Reader Service No. 116.*

• VG Isotopes, specialists in isotopic and elemental mass spectrometry, has announced a new analytical research service for scientists, researchers and technologists. VG Isotopes Analytical Service Group has at its disposal an array of techniques including inductively coupled plasma mass spectrometry (ICP-MS), glow discharge mass spectrometry (GDMS) and thermal ionization mass spectrometry (TIMS). The analytical service should be particularly attractive to those who would not otherwise have access to such advanced instrumentation, or need only occasional access to such equipment. *Reader Service No. 117.*

• The Gilford Response range of UV/Vis spectrophotometers from Ciba Corning has now been extended to three separate models. Common to all models is: an autoranging photometer; transverse sample compartment; high resolution graphics; data storage facilities and a 16-bit resident computer. The basic Response is supplied complete with a single cell holder and programs for: wavelength scanning; kinetics; time scanning; multi-wavelength analysis; standard curves and sample read. The second unit has an automatic six-position cell holder as standard and has the capability of adding gel scanning, rapid sampling and a six-position thermostet cuvette holder for electronic control of sample temperature. The most advanced Response has two disk drives for archival data storage, together with extended software applications. *Reader Service No. 118.*



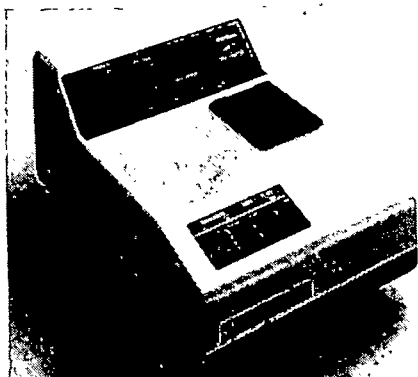
Response spectrophotometers.

PRODUCT REVIEW

ADVERTISEMENT

• The new Gallenkamp Visi-Spec is a low-cost spectrophotometer for measurements in the visible spectrum. It is suitable for general purpose measurements where cost and reliability are important. The complete optical system is microprocessor controlled. There are only four push button keys and it automatically goes through a three-point wavelength calibration check every time it is switched on. Standard 10-mm cuvettes or test-tubes are accepted.

Reader Service No. 119.



Gallenkamp's Visi-Spec.

• The Oriel Scientific DRTA-9 integrating sphere accessory for the Perkin-Elmer Lambda 9 UV-Vis-NIR spectrophotometer is a six-inch diameter sphere which can collect either scattered light from turbid solutions or reflected light from solid samples. This accessory expands the usefulness of the Lambda 9 beyond its standard application for homogeneous, translucent solutions and makes it possible to perform quantitative analysis of solid materials used in solar and optical component research. The analytical chemist and life scientists are offered qualitative analysis of turbid solutions, NIRA and Kubelka-Munk functions without extensive sample preparation. The DRTA-9 fits entirely inside the Lambda-9 sample compartment cavity and is easily installed by the system operator.

Reader Service No. 120.

• Kontron Instruments' Uvikon 860 UV/Vis spectrophotometer offers a combination of intelligent electronics and proven optics, with the intelligent electronics making full use of a video display and RAM curve memory for entering parameters and post measurement calculation. Each parameter is presented to the operator, together with its possible value. The Uvisoft keys which are software defined function keys appear on the video display



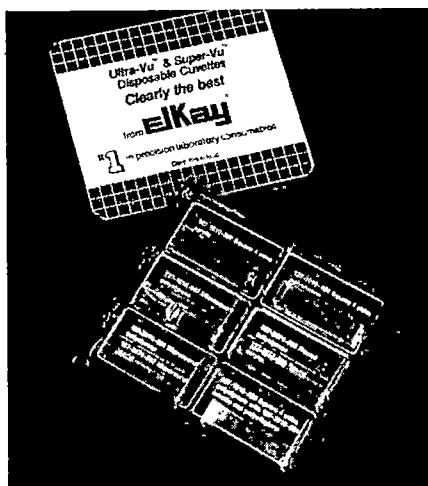
The Uvikon 735LC.

allowing the Uvikon 860 to be very user friendly. In addition to the normal measurement modes, 'fix' and 'scan' the Uvikon 860 has programs for enzyme kinetics, end-point analysis and absorbance versus time. By using a personal computer the operator also has access to the internal commands of the Uvikon 860 and personalized programs can easily be written to suit the operator's own application. In addition, the Uvikon 860 allows complete sets of method parameters to be stored in permanent memory, ready for fast set up of routine conditions.

Reader Service No. 121.

• Cuvette sample packs are being offered by Elkay Products Inc. free of charge to laboratories wishing to evaluate the company's six different disposable cuvettes. Precision moulded in UV-inhibitorless polystyrene or acrylic copolymer, the cuvettes are capable of averaging 70% light transmittance at 340 nm. Each cuvette is packed in particle free, shrink wrapped reusable polyfoam trays.

Reader Service No. 122.



Free cuvettes from Elkay.

• Beckman's data capture software for use with DU-7 and DU-50 UV/Vis spectrophotometers is compatible with the IBM PC series of desk-top computers and features colour graphics with easy colour selection. The software allows single-wavelength, scanning, kinetics and gel scan data to be transferred to the computer and conveniently stored in labelled files on standard disks. With the DU-7 spectrophotometer stored data can be transferred bidirectionally so that data from disk may be viewed and manipulated on the graphical video display of the DU-7. With the DU-50 series stored data can be displayed quickly on the computer's monitor with overlay of curves in different colours, and scans can be added and subtracted to give accumulated results or difference spectra. All data are stored in a standard file format which is directly compatible with such popular general software as Lotus 1-2-3. With Lotus, data can be entered into a spreadsheet presentation for carrying out complex calculations and data manipulation.

Reader Service No. 123.

Geological Sciences Series, Vol. XIX Stanford University

New Publication.

Basic-Hypo; A Basic Language Hypocenter Location Program User's Guide

Jorge Mendoza
Dale Morgan

An efficient BASIC language computer program for the location of hypocenters of local and regional earthquakes, is implemented on a 5¼" diskette.

The publication includes the source code and it can be typed in computers, like Commodore 64 (TM), Radio Shack TRS 80 (TM), Hewlett-Packard Series 80 (TM), Apple II family (TM), IBM PC (TM) and all PC compatibles and any other computers that run Microsoft Basic (TM).

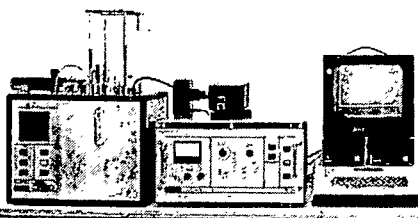
The diskette included in the publication is MS DOS compatible.

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Stanford, California 94305
Att. Margaret Muir

• For those involved in the study of rapid reaction intermediates, Hi-Tech has introduced the SFL-43 stopped-flow/multimixing flow module as an additional capability to the SF-4 spectrophotometer system. This flow module can be used in two modes in the study of reaction intermediates or just simply for high performance stopped-flow work. In the sequential mixing mode, two stable solutions are mixed to form a short-lived intermediate which in turn is mixed with a third reagent after a pre-set delay as short as 5 ms. The second reaction is studied by stopped-flow spectrophotometry. The preparative quench flow made enables a fast chemical or biochemical reaction, that



SFL-43 augments the SF-4.

lacks a suitable chromophore, to be analysed by an intrinsically slow technique such as NMR spectroscopy. Initiated by mixing, the reaction is stopped after a pre-set delay by mixing with a quench solution and the product is then collected for analysis. By varying the delay a series of values may be plotted to determine the reaction rate. The flow circuit is automatically purged by solvent before the run, thus eliminating cross-contamination.

Reader Service No. 125.

• Bio-Rad's new Model 1745 fixed-wavelength UV monitor is a highly sensitive detector suitable for both HPLC and open column applications. In standard form, the instrument is able to monitor UV absorption at either 280 nm or 254 nm — the most popular wavelengths for protein, pharmaceutical and nucleic acid applications. For peptide studies a low-cost zinc lamp can be added to provide detection at 214 nm.

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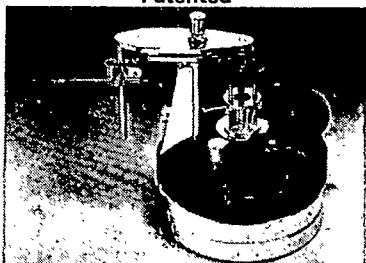
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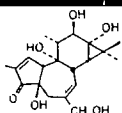


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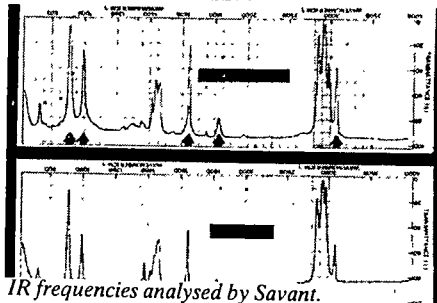
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Reader Service No.11

action rotary control allows the change from reference to sample to be made easily. The S107 is fitted with a fan cooling unit which allows the cuvettes to be maintained at closer temperature tolerances. In addition to manual operation, the S105 can be used in a semi-automatic mode. It can also be provided with a scanning facility by the addition of a small electric drive unit and, when coupled with a chart recorder, this instrument can make fully automatic scans.

Reader Service No. 127.



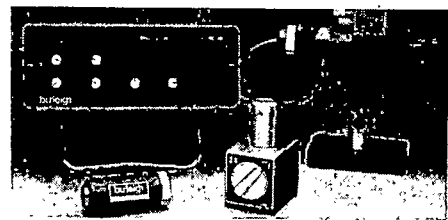
IR frequencies analysed by Savant.

• An audiovisual training program on the interpretation of infrared spectra, written by J.H. van der Maas and E.T.G. Lutz has been published by Savant Audiovisuals and John Wiley. Composed of eight lessons which systematically cover the major IR group frequencies, the package contains 123 slides, four tapes, 10 course books, an answer book and 100 correlation tables. It is illustrated with high quality spectra of known compounds and unknown materials for student practice. This educational package (IR 1000) complements Savant's other infrared audiovisual programs which are concerned with techniques, instrumentation and applications. They cover infrared quantitative analysis (IR-101), solid sample handling for infrared spectroscopy (IR-102), infrared internal reflectance spectroscopy (IR-103) and computerized infrared spectroscopy (IR-104).

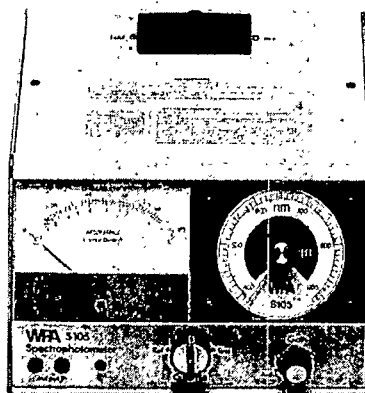
Reader Service No. 128.

• Burleigh Instruments, Inc. has introduced two new mirror sets which extend the spectral range of the company's SA-800 and SA-200 Series optical spectrum analysers. The new mirror sets cover the important wavelength regions of 800-900nm and 1.06 μ m. Burleigh spectrum analyser systems are modular and feature an x, y, theta and phi adjustable mount for complete control over the alignment process. Each system is delivered with spectrum analyser, controller, detector, beamsplitter, mount and bases. The analysers are being used extensively as a diagnostic for mode-locked YAG systems.

Reader Service No. 129.

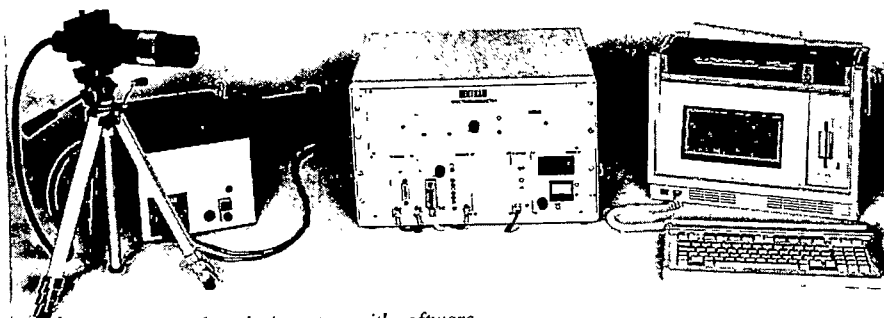


Burleigh's RC-46 controller with accessories.



WPA S105 spectrophotometer.

• The S105 and S107 spectrophotometers from Walden Precision Apparatus are designed to cover the UV and IR ranges, operating between 380 and 920 nm. A slow motion wavelength selector control with a large scale and pointer is incorporated in these instruments. The scale is marked at 5-nm intervals and the approximate colours of the visible spectrum are shown to ensure accurate wavelength selection. The symmetrical optical system achieves a bandwidth of less than 5-nm and the illumination is provided by a special tungsten filament lamp which gives a continuum of emission from about 300 nm to well into the infrared spectrum. Both spectrophotometers accept standard glass or plastic 10 mm cuvettes which are shielded from extraneous light. A quick



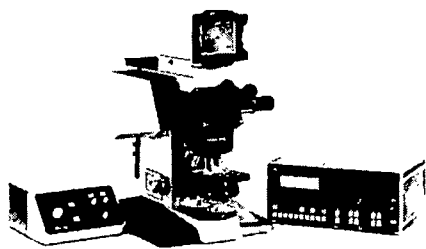
The Bentham spectral analysis system with software.

- Bentham's new modular spectral analysis system can be used over the wavelength range from 200nm to 30 μ m. The system can as easily be configured for accurate colour measurement of displays as it can be for measurement of transmission and emission in the ultraviolet and infrared regions. Light sources, detectors and electronics including lock-in amplifiers are available, while system control is facilitated by the use of the IEEE/488 interface bus (GP-IB, HP-IB). Popular applications include colour measurement, for which calibrated standards are supplied, measurement of spectral loss in fibre optics and monitoring of plasma etching processes. A software package available for HP-85 and some other popular desk-tops perform general spectral measurement and fibre loss.

Reader Service No. 130.

- Polyspec, is a new microspectrophotometer from Reichert-Jung. It is a modular system comprising a basic system for measuring transmittance (extinction), reflectance or fluorescence which can be upgraded to microspectral analysis or to an integrated complete microscope measurement system with automatic scanning control. Providing high sensitivity and reproducibility, this new system permits observation of the measuring diaphragm during measurement by employing the synchronized dual chopper principle. The Polyspec has a choice of two operating modes — either the HP (high precision) chopped mode taking 140 measurements per second, or the HS (high speed) d.c.-mode at 1,000 measurements per second. The control unit employs 2 Z-80A microprocessors, it can be fitted with up to eight plug-in dedicated applications program modules, and has a liquid crystal display capable of graphics presentation. The Microspectrophotometer may be interfaced to a computer for programming and data processing and to a printer-plotter.

Reader Service No. 131.



Reichert-Jung's Polyspec.

- Philips Analytical has developed two new low-cost ultraviolet/visible spectrophotometer data systems based on the PU8800 series of instruments. The PU8800 DS system, designed for scanning work, consists of the high performance double beam PU8800 spectrophotometer, a computer customized for the laboratory environment, and a scanning software pack. The software permits spectral display, overlay and expansion using a zoom facility. Spectra, each containing up to 7,000 data points, can be stored on disk, together with optimized display parameters and PU8800 scan conditions. The second system, PU8800 DSK, is specially designed for kinetics and comprises a PU8800 spectrophotometer, computer, six-cell changer and an advanced kinetics software pack. Features include absorbance against time plots and rate calculation with linearity check. Printouts of rate, activity and purity can also be obtained. K_m and V_{max} values can be calculated by either a direct linear plot or regression analysis, and the plots and results displayed in a few seconds.

Reader Service No. 132.



The PU8800 UV/Vis spectrophotometer.

- A completely new analytical transmission electron microscope from Carl Zeiss (Oberkochen) incorporates a fully integrated electron loss spectrometer. This gives the Zeiss EM902 the capability of performing Electron Spectroscopic Imaging (ESI), providing significant improvements in contrast, and high resolution elemental mapping over conventional techniques. Using ESI it is possible to analyse and image light elements not accessible to X-ray microanalysis with sensitivities and resolutions not even theoretically attainable by X-ray spectrometry.

Reader Service No. 133.

- The latest issue of Finnigan MAT publication Spectra covers clinical applications of mass spectrometry and includes articles on organic acidurias in children, use of GC/MS in clinical toxicology, eicosanoid mass spectrometry and steroid profiling by GC/MS.

Reader Service No. 134.

- The Kratos Analytical MS80 is a high performance medium resolution mass spectrometer designed for both routine operation and advanced research problems. It features double focusing forward geometry that incorporates hexapoles, with advanced source construction and a novel magnet design that gives outstanding sensitivity maintained across the range of resolving power. Exceptional capabilities are available in quantitative selective ion monitoring, at picogram levels and below, of materials in complex mixtures. A comprehensive range of inlet systems includes gas chromatography, with facilities for packed and capillary column work. Fully programmed digital logic and display units give push-button control of major instrument functions. The fast scanning RF magnet is of particular use in capillary column GC/MS, both in terms of its fast scan speed and also in its speed of switching in magnetic selectors ion monitoring mode. The MS80 can be provided with an LC/MS inlet utilizing the Thermo-spray mode of operation.

Reader Service No. 135.

- A new mass spectrometer for automatic ^{15}N analysis has been introduced by VG Isogas, the ANA-SIRA (for Automatic Nitrogen Analyser-Stable Isotope Ratio Analyser) mass spectrometer system. The ANA-SIRA can automatically analyse both solid and liquid samples for ^{15}N and total N with sample sizes as low as 5 μg N at natural abundance. This combination of sample preparation and analysis enables fast (5 min per sample), reproducible (± 0.0005 atom % ^{15}N), and automatic analysis in the fields of biomedical, agricultural and environmental research. An ANA Interface upgrade kit is available for all VG Isogas mass spectrometers.

Reader Service No. 136.



LeCroy 9400 Digital Oscilloscope

- Fast logging of waveforms data is particularly useful when signal availability is short, for instance in electron spin resonance experiments and laser spectroscopy measurements. A segmentable 32K bit memory in each channel of the new LeCroy 9400 Digital Oscilloscope enables logging of successive events on trigger command. This allows very fast acquisition of waveforms, since no transfer of digitized data to external devices such as a computer or disk is needed. Up to 250 waveforms of 125 words can be logged well within 50 ms at high time-base speeds in the acquisition memories, or 125 waveforms of 250 words within 25 ms, or as fast as 8 waveforms of 2,500 words within 2 ms.

Reader Service No. 137.

Graduate mobility in the United Kingdom

from Richard Pearson

Increasing interest is being paid in the United Kingdom to longer-term graduate careers; several cohort studies following graduates from 1980 and later years are now under way or are being planned.

Job prospects and employment patterns of new qualifying graduates are seen by many as an important indicator of higher education's "success" and relevance to industry. Others argue that they say nothing about lifetime returns from higher education which should not be exclusively job-related. Nevertheless, by providing an easily quantifiable indicator, trends and comparisons based on the first destination statistics will be made. While they provide a valuable barometer of initial labour market success, they are not a definitive statement of longer-term employment prospects. A typical working life lasts forty years or more and in the United Kingdom there are more than six million job changes each year. Evidence about employment status six months after graduation can provide only a starting point for assessing graduate careers.

In providing a broader context, a recently published study¹ of graduate mobility based on the experiences of 280 em-

ployees, original employer five years later. This may be in part explained by the low recruitment levels in this sector in the early 1980s resulting in a shortage of experienced graduates three to five years later which in turn encouraged mobility between employers. By contrast, only 26 per cent of those recruited by chemical companies in 1980 had left their initial employer in the following five years. More general evidence suggests that the retention curve flattens after five years or so, and that after 10 or 15 years probably 30 per cent of graduates are still with the same employer.

These studies show only general trends. The best source of data on the mobility of individual graduates remains the now dated study by the Department of Employment of 12,000 students and their careers and mobility over the seven years to 1977. That study showed that men graduates were more mobile than women graduates, both between their occupations and type of work, but that women were more likely to move between employment sectors. The main motivation of graduates in changing jobs was the "job interest" and "level of responsibility offered" but the prospects of a good salary and promotion assumed increased importance as their careers developed. Women's salaries were about 20 per cent below those of men after seven years, a difference only partly explained by the higher incidence of part-time work among women. The highest salaries were, in 1977, paid to graduates in social studies or engineering, the lowest to arts graduates. Managerial jobs in industry and commerce tended to be the best paid, while school teachers were among the lowest paid. It was also found to be less rewarding financially seven years after graduation to have studied and gained a postgraduate qualification than to have entered employment directly and gained work experience. This study is due to be repeated next year, based on the cohort graduating in 1980.

The most recent major survey of graduates' early careers has been that being undertaken by the Council for National Academic Awards (CNAA) which is surveying 10 per cent (2,600) of graduates who received CNAA degrees in 1982. The students were surveyed in 1983, 1984 and 1985, allowing a three-year profile to be drawn up of their early careers. The results of the 1983 survey reveal a complex pattern of transition over the first year into the labour market. They show significant numbers entering, for

example, part-time or temporary employment and one in six changing their job within the first year. In many of the "vocational" subjects such as pharmacy, nursing and business studies, the graduates were in their preferred job, but 40 per cent of all graduates and 60 per cent of scientists were looking for "something better". Nearly half the scientists felt over-qualified for the job they were then doing and one in six scientists had already had two or more jobs in their first year after graduation. A further 41 per cent had considered changing jobs. Nearly a year after graduation, the average salary for a scientist in 1983 was £4,867, considerably below that of engineers (£6,358) and maths/computer scientists (£6,354) but similar to

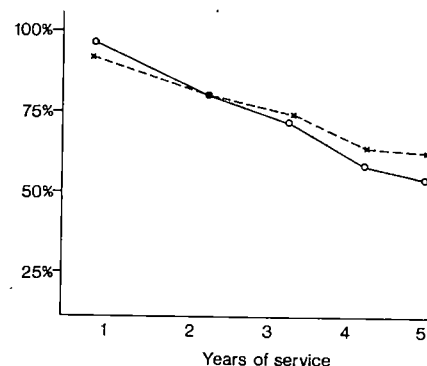


Fig. 1 Graduate retention. Taken from ref. 2.

that for social scientists (£4,865) and just ahead of the arts and humanities graduates (£4,672). The average salary across all subjects was £5,058. More than one in four of the CNAA scientists was unemployed ten months after graduation which is rather more than at the six months stage.

For graduates, like all other first time entrants into the labour market, the first job is only the first part of a career, the early years of which are likely to see several changes of job and possibly of employer. The first year for many can be a particularly difficult year of transition, especially, as in 1982, in times of a depressed labour market. The first destination statistics provide one snapshot, and give a limited performance measure; they do not however provide a complete picture of life after higher education.

1. Parsons, D. *Graduate Recruitment and Retention 1980-84*. HMS, 1985.
2. CNAA Graduate Survey. Interim Report, 1985.

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Graduate retention 1974-79 and 1980-84

Retention dates	Year of cohorts*				
	5th	4th	3rd	2nd	1st
1974-79 cohort	54	59	71	84	96
1980-84 cohort	58	66	72	84	94

* 1st = most recent, that is 1979 and 1984 respectively.

ploying organizations showed that nearly 40 per cent of graduates recruited in 1980 were no longer with the same employer five years later. As Fig. 1 shows, there is a fairly standard decay curve, with 34 per cent of the 1981 cohort and 16 per cent of the 1983 cohort having left their initial employer by 1985. In addition, most graduates who have stayed with a single employer will also have changed jobs over this period and some will also have moved geographically. What is particularly interesting about the 1985 study is the comparison that can be made with the results of a similar study in 1980. The time span for each was five years, and each covered a similar economic cycle from a low to a high point of economic activity. Despite the fact that the recession of the 1980s and the contraction in job opportunities was far more severe, retention rates followed remarkably similar patterns (Fig. 1). Despite this consistency over time, there were differences between employers from different sectors. The sector with the highest loss was engineering, where 60 per cent of the 1980 graduates were no longer with their

Answering the \$144 billion question

The US Congress will probably have settled the course of the world's economy for a decade in the past few days of hectic pre-Christmas legislation. It may not have the leisure in which to repent.

ONE thing about the future is now certain. The US federal government's deficit for the financial year beginning in October 1986, whimsically called "Fiscal 1987", will be not a penny more than \$144,000 million, just about two-thirds of the likely deficit in the current financial year. And how can anybody be sure of that in such an uncertain field? Because the Congress will this week have passed a law to that effect and because President Reagan will not have dared to veto the bill for fear that the federal government will otherwise be unable to borrow the funds it needs to pay its bills, pre-Christmas salary cheques due to federal employees among them. More than this, the Congress has also decreed a linear decline of the annual deficit until, in fiscal 1991, it vanishes altogether. It is as if a government had decreed that the incidence of murder, or of AIDS, will decrease in four equal instalments to vanishing point.

Naturally, neither the Congress nor the administration in Washington is as naive as these appearances suggest. The notion that the US government might first bind itself to make the deficit vanish and then buckle down to the task of reaching that previously unattainable goal has been around since the early summer. It has gathered force since September, when the members of President Reagan's political party in the US Senate felt themselves betrayed by the President's agreement with the Democratic Speaker of the House of Representatives which had the effect of killing off the Senate Republicans' plan for squaring the circle of the budget deficit. The notion provided marvellous material for newspaper columnists with opinions on the US constitution, for it means that Congress should surrender its right to legislate how public money is spent to a person whose only qualification for public administration is the possession of a hand calculator. But now, when the administration needs to increase its power to borrow money above 2×10^{12} for the first time, the Gramm-Rudman bill (named after Republican senators from Texas and New Hampshire respectively) has inevitably attached itself to the bill that would provide the borrowing authority needed to keep the United States afloat. By now, President Reagan will probably have both his borrowing power and the threat that next year's budget will be determined not by priorities but by simple calculations of proportionality.

Basic research in the United States will be one of the early casualties. This is how that will come about. Next year's deficit has been pegged at \$144,000 million, and the administration has said that it will live within that limit. But the administration has also said that it will increase military spending by 3 per cent while not increasing taxes. Since large chunks of public spending (salaries and social security, for example) are fixed by law, the money will have to come from the government's dispensable spending, science and education included. This, no doubt, is why there is so much anguish about the preparations for the 1987 budget, due to be made public before February next year. It is hard to believe that the rising trend of research spending over the past five years will not be reversed next year, in the pursuit of a deficit of a mere \$144,000 million. But there may be worse to follow, for if the Congress and the administration fail to agree on a budget within the law, there will be *pro rata* reductions of all adjustable spending programmes. So next October, agencies such as the National Science Foundation and National Institutes of Health will be robbed of funds they had planned to spend, but

to a degree that will be known only a few days before the financial year begins. And there is an even more immediate threat; the same legislation specifies a cap for the budget deficit for the current financial year, now nearly a quarter gone. If, on 1 February next, it seems that spending will exceed \$176,000 million, which is almost certain, there will be an immediate cut across the board (which will nevertheless not exceed \$11,000 million). Will research grants be cut individually, or merely postponed? And with what effect?

The most helpful analogy of this way of conducting a great nation's financial business is the old recipe for extracting the teeth of dentist-shy children by tying a string to the tooth and to a door-handle, allowing the tooth to be extracted by the first person to open the door. In the last resort chance will be substituted for reason. It is a prescription for doing most damage to the public spending programmes which most of all depend on continuity and the pursuit of long-term objectives. It will be a great waste if the Reagan administration, with its creditable record of support for basic research over the past five years, should not put the enterprise into reverse, either in the process of meeting the targets Congress has decreed or in the inevitable ruckus about spending that is bound to erupt in 1986 (which is an election year).

Not that the objectives that the Congress had set for the government are invalid. The US budget deficit has been a literally mounting scandal for the past four years, causing endless damage in the United States and elsewhere. To cover its deficit while trying (successfully) to contain inflation, the US government has had to borrow funds that would otherwise have gone into private investment in the United States. So, until recently, the dollar has been overvalued relative to other currencies and the United States has been saddled with a deficit in its external trade numerically almost as large (\$140,000 million or so this year) as the budget deficit. As a consequence, the clamour had gone up that traditional industries being driven out of business should be protected against competition which is unfair only in the sense that it is caused by the budget deficit, and is thus artificial. As yet, the threat to world trade has been contained, largely because the administration has stoutly set its face against the erection of trade barriers, yet there is now alarming evidence that world trade is in serious trouble; OPEC cannot sell its oil, nor can the International Tin Council sustain the price of tin. So there is a danger that developing countries will be even less able to pay their debts by means of exports and that industrialized countries will further languish.

Gloom

If the gloomy forecasts that inflation has now given way to deflation should prove correct, it will then be plain that the Gramm-Rudman legislation has arrived at just the wrong time. For then, monetarists will be required to behave as if they were Keynesians, pumping money into the economy even if they have to print it, applauding budget deficits as the simplest way of curing an economic evil potentially as damaging as the inflation that seems only just to have been moderated. It seems inevitable that there must be some switch of policy along these lines long before the timetable for making the US budget deficit zero. Sadly, much damage has been done in getting this far. □

No pigs in pokes

A British dimension to the US Strategic Defense Initiative is not necessarily a bad thing.

THE trouble with the Strategic Defense Initiative (SDI) is that it means all things to all men. To Mr Reagan, it ensures immunity from other people's hostile missiles, but to Mr Gorbachev it is an abomination. Mrs Margaret Thatcher, the British Prime Minister, is somewhere in between. Through her, the British government has insisted that SDI is a research programme only, and that there is no question of deployment unless the owners of hostile missiles are consulted. Mrs Thatcher has also taken the lead in Western Europe in saying that influence of this kind requires collaboration, but only if the price is high enough.

How well does last week's agreement between the defence ministers of Britain and the United States match these good resolutions? Nobody can be sure. Virtually the only provision of the document made public is that which specifies that the substance of the agreement will be kept secret "in perpetuity". There will never be an opportunity for telling what costs, and what prizes the participants can expect. For all that can be told, the agreement may consist only of a single sheet of paper binding the participants merely to declare they have agreed to collaborate on SDI, but saying nothing else (together with the bit about perpetuity). So what should such an agreement contain? Even those who believe that Britain should not take part have an interest that the agreement should be equitable.

Technically, there are several important issues, of which the chief is whether SDI will be a stimulus to economic innovation. The argument is simple, but also simplistic. Spend money on military innovation, and you cannot fail to generate economically worthwhile innovations. So the more technical effort in British defence laboratories, the more prosperous British civil industry will become. There are two obvious objections. First, Japan, the most successful source of economic innovation, supports only a small military establishment. Second, Britain has for four decades spent conspicuously on defence research, but has been woefully inadequate at converting military into civil innovations. Whatever the secrets of last week's agreement, there is no chance that economic benefits will flow without structural changes in the relationship between military and civil research.

Worse, in British (but not American) conditions, the involvement of industry with powerful customers for knowledge (as SDI will be) can have the opposite effect; in retrospect, the dependence of the British telecommunications industry on the old public monopoly of British Telecom, far from making it competitive with free-standing industries elsewhere, actually made it harder for British companies to hold their own in world markets. At this stage, arguments about proprietary rights to inventions are academic.

So the long-term commercial benefits to Britain cannot be large unless there is structural change. Will the short-term cash (there has been talk of British organizations winning 7 per cent of the total) be of assistance? Here again, the arithmetic is clouded. If there is more money, some who might have emigrated may stay in Britain; the other side of that coin is that close acquaintance with SDI may tempt others who would have stayed in Britain to cross the Atlantic. But the most likely (and sombre) course of events is that great skill will be locked up in a programme of little economic value. The chances that people will pick up skills they never had, and will afterwards deploy them in civil applications, are small.

Technically, SDI is more likely to be a diversion than an opportunity. Politically, given the point from which the British government starts on strategic issues, it is on more sure ground. At some stage, there is bound to be an argument about the rights and wrongs of deployment. Most probably, if SDI yields anything of value to military technology (early warning is probably inevitable), there will be pressures to deploy what machines there are before the other side has been consulted. That is when

Mrs Thatcher or her political heirs will be able to cash the political cheques they will earn in the next few years. The question, and perhaps the darkest cloud over last week's agreement, is whether British governments will keep their independence and their nerve over the whole decade ahead. □

Australia's grudge

The McLelland report seems a fair complaint. What can be done?

THAT Australia should have been up in arms about the uses made by the British government of the deserts of Western Australia for testing British bombs three decades ago is natural and understandable. Last week's report on more than two years of a judicial investigation of the matter (strictly by a Royal Commission set up by the government of Australia) has failed to substantiate the more lurid tales told in evidence, but is otherwise fair. The chief recommendation, that the British government should somehow compensate Australia for the damage done, perhaps by cleaning up the radioactive mess left behind, is on the face of things a reasonable demand. But there is a danger that relations between the two governments will deteriorate into an unseemly squabble unless both of them acknowledge how much has changed, technically and in their political relations with each other, since the days when the Prime Minister of Australia, Sir Robert Menzies, was one of Sir Winston Churchill's closest buddies.

With the lapse of thirty years, it is hard fully to appreciate that even technical people could have been as naive as those in charge of the early nuclear tests about the dangers of radioactivity, both ionizing radiation produced directly from exploding fireballs and radioactive fission products or other radioactive isotopes produced indirectly, by the irradiation of material in the environment. To be sure, by 1945 there was a growing body of knowledge of the problems. The effects of X rays had been carefully studied, while the Manhattan project had thrown up a substantial understanding of the effects of external radiation by other materials, neutrons for example. The destruction of Hiroshima and Nagasaki eventually yielded crucial data about the biological effects of acute doses of external radiation. But of necessity, the effects of small doses (sustained by people some distance from the explosions) accumulated only as the years went by. So, by the time of the United States conference on the Peaceful Uses of Atomic Energy exactly thirty years ago, it was possible for the then chairman of the then US Atomic Energy Commission, Mr Lewis Strauss, to insist that there must be some kind of threshold dose of radiation below which no biological damage would be done. While Mr Strauss may have had a special flair for resisting unwelcome ideas, he was by no means untypical of those with influence in the 1950s. And only about that time did even the critics of nuclear weapons testing begin to accumulate telling evidence of the kind that eventually brought tests of nuclear weapons in the atmosphere to a halt.

The other important change that has come about in the past thirty years concerns Britain and Australia, then bound together both by the warm sense of having worked successfully together in a good cause (the Second World War) and, less easily, by the old ties of empire. It is hard now to reconstruct the familiarity of that relationship, although it seemed as natural to many Australians as it seems to have done to Menzies that the British should have been given more or less free range of the testing grounds in Australia. That the use they made of their licence may have been, by present standards, imprudent is amply demonstrated by last week's report. Much the same was happening then and earlier at the US testing grounds in the Pacific (see p. 502). The most urgent need now is that the British government should openly acknowledge the likelihood that much of Judge McLelland's argument is correct. To do that need be no shame (nor entail the A\$100 million talked of). To fail to make proper acknowledgement would be unjust, and make still more distant the relationship between Britain and Australia. □

SDI

Star wars research not up to scratch?

Victoria, British Columbia

THE US government is not rigorously applying the usual criteria for the assessment of scientific projects to all research proposals to the Strategic Defense Initiative (SDI). This is the nub of the allegations against SDI levelled by David Parnas, Landsdowne professor of computer science at the University of Victoria.

Parnas acquired a certain notoriety when, last June, he resigned from the computing panel of the SDI project, to which he had been appointed an adviser a

There's no money in crime—go for SDI contracts.



few months earlier. He has since become one of the most outspoken critics of SDI on technical grounds.

Parnas is due to give evidence to a sub-committee of the US Senate's Armed Services Committee this week. He says that the essence of his complaint will be that scientists seeking research contracts have discovered that "when the decisions are made by people who have a mission, they tend to give the money to the people who make the biggest promises".

Parnas is especially critical of those at universities who have taken SDI money while knowing that the project will not succeed. His resignation letter in June said that he did not believe that "further work by the panel will be useful; I cannot, in good conscience, accept further payment for useless effort".

To the criticism (by SDI) that the computing problems would have been less conspicuous if those responsible for them had made greater use of artificial intelligence (AI), Parnas says that he has been watching the advocates of AI for years and has remained unconvinced. "They make extremely false claims. They solve very simple problems, but when you look at them closely, you find that the methods do not generalize."

To Parnas, the chief problems confronting SDI are that it will never be possible to test the system properly, while the researchers will not be able to define the be-

haviour of the attacking missiles with sufficient clarity.

In his own field, Parnas says that software is the last part of a system to which designers pay proper attention. Although it may be easy to "get the bugs out of" the mechanical part of a system, design flaws in the software will come to light only years after it has been put into operation. Yet the task facing the SDI software will be formidable; on one estimate, the system will need no fewer than 10 million lines of code.

Parnas guesses that, in an SDI system, there will have to be a computer on board every satellite, in the first instance to process the raw data. "Even if you are going to do the computing in a centralized system, you are going to have to do some

computing on the satellites to reduce the amount of data you have to transmit." Yet it will also be necessary for the satellites to control their own sensors and some weapons.

Parnas is convinced that the software needed for SDI is so complicated that it cannot be expected to function reliably. He argues that there will have to be systems for handling the data from the sensors, and for computing the trajectories of hostile warheads, distinguishing them from decoys. Then it will also be necessary to determine the paths of retaliatory missiles, and to decide when they should be fired. "Software", says Parnas, is the glue that holds such systems together." He notes that both the Apollo Moon programme and the space shuttle have been delayed by software problems. He is not against military research programmes, and remains a consultant for the US Navy. Yet he is resentful that money should be channelled into what he considers to be the wasteful SDI programme, as well as the wasteful use of manpower and of computers that will be involved. **Bill Johnstone**

SDI

British role is still unclear

Washington

THE long-awaited agreement signed last week in London on British participation in the Strategic Defense Initiative (SDI) should allow British researchers to bid for a "significant" proportion of the \$26,000 million the Reagan administration wants to spend on the programme over its first five years, according to British officials. But despite British efforts to secure a commitment from the United States to at least \$1,500 million-worth of contracts, no guarantees of specific sums are mentioned in the agreement.

British/US scientific collaborations have in the past been marred by accusations that Britain has failed to secure adequate returns. The most contentious aspects of last week's agreement, those relating to intellectual property rights, were not made public by British Secretary of State for Defence, Michael Heseltine, and US Secretary of Defense Caspar Weinberger, and the exact terms are likely to remain secret. Instead, Weinberger and Heseltine agreed that collaboration would be as "full and fair" as national laws permitted, and that there would be the "fullest possible retention of benefits of participation".

When dealing with domestic companies, the US Department of Defense (DoD) waives its rights to patents arising from basic research that it supports, with the proviso that it will not subsequently have to pay royalties to the developers. It is unclear whether it will adopt the same position for foreign companies.

Closely related to the question of intellectual property rights is that of security

classification. While the majority of SDI contracts let so far have been classified, those originating from the SDI organization's innovative science and technology office — some 5 per cent of the total by value — are unclassified, and the director of that office, James Ionson, has said that unclassified research projects will not be classified retrospectively. US specialists caution, however, that the limits to permissible commercialization of research funded by DoD are uncertain at the best of times, and depend greatly on how strictly the Pentagon chooses to enforce particular cases.

Another problem for British companies is likely to be that DoD as a matter of deliberate policy will often divide up or "compartmentalize" different sections of strategically sensitive research for security purposes; the result is to make integrating the results of a research project difficult for the individual investigator.

British officials stress that participation in SDI research does not imply an unfettered commitment to building and deploying a ballistic missile defence; Britain says it stands by an agreement reached a year ago between Prime Minister Margaret Thatcher and President Reagan, in which the two leaders agreed that any deployment of such a system would be subject to negotiations.

British participation in SDI will be coordinated by a new SDI participation office established within the Ministry of Defence in London. Areas where collaboration is expected include architectural design studies, optical computing and electronic materials. **Tim Beardsley**

French research planning

How will the future come about?

PERHAPS the French Revolution so divided and politicized the nation that every now and again France seems to need to pray together, to demonstrate its solidarity. This is the spirit in which researchers and industrialists met at the National Colloquium on science and technology in 1982, and this was also the spirit in which the same groups met again at the end of last month, at another grand mass — one praying for the future.

The meeting, "Prospective 2005", was jointly sponsored by the Centre National de la Recherche Scientifique (CNRS) and by the Commissariat du Plan (le Plan for short), which together had spent a year on the preparations. In many ways, the meeting marked le Plan's return to the public stage. Founded after the Second World War, le Plan became famous for its planning of France's spectacular economic rebirth during the 1950s. More recently, it has been somewhat moribund, suffering an uneasy relationship with, and neglect by the socialist government until, it seems, le Plan discovered technology.

The main fruit of the discovery so far seems to have been last month's colloquium on technological France twenty years ahead. But, unfortunately for le Plan, the general feeling was that the year of study by expert groups, and the voluminous documents they presented to the colloquium, amounted to a flop.

A report on communications, for example, was described by one communicator as "arid"; no doubt he was as aware as the other participants of the tremendous political debate then (and still) raging outside on the prospect of a fifth (private) French television channel run in collaboration with Italian and British interests and the future use of the first (and French) European direct broadcasting satellite to be launched next October.

The formal documents on the future of medicine similarly failed to take much account of the potential contribution of genetic engineering, but one of the colloquium rapporteurs dismissed — with venom — the notion that microgravity (as in a space station) might be of great importance in materials processing.

The rights and wrongs of these issues are certainly arguable. The future of French television was firmly outside the meeting's terms of reference. Less glamorous techniques than genetic engineering (such as halting smoking) might have more impact on public health in 2005 than any monoclonal magic bullet. But materials processing in space does occupy a large proportion of French research ministry funds devoted to work of this kind. One aggrieved representative of French industry complained at the meeting that the research ministry's funds devoted to space-based and land-based special pro-

grammes in materials science are in the ratio 4:3 (FF80 million against FF60 million). One consequence of this vein of realism, as the French would call it, was the opposite of what might have been expected of the nation that nurtured Jules Verne: the colloquium made the future seem a little dull.

The exception was the dramatic presentation of the future of unemployment in 2005. We are moving away from a civilization of pain and labour (*civilization de la peine*), the colloquium was told, to one of *civilization de la panne* (implying breakdown), a more flexible way of working than at present. The mere labour of the workers would no longer be needed, the colloquium was told; twenty years from

now, there would be 100,000 robots at work in factories, 25 times as many as at present. Before then (ten years from now), there would be an electronic workstation on every office desk. By 2005, retraining would occupy 10 per cent of people's working hours, which would by then be reduced by a quarter. The urgent need, the conference was told, is that institutions and trades unions should worry about the career patterns of the future.

This uplifting theme evoked a sour note. The intended massive increase of education and of retraining is a policy "of the elite for the elite" according to one who should know, the director of an employment office in north Paris with 3,500 unemployed people on its books. He doubted whether ordinary people would be able to cope with what the future, even as described, would expect of them.

Robert Walgate

UK medical research

MRC defends core of excellence

UNDER continuing financial pressure, the UK Medical Research Council (MRC) intends fully to protect the best jewels in its crown, but to throw out those that are substandard. Speaking in London last week, Sir James Gowans, secretary of MRC, said that only then would it be possible for the council to afford much needed equipment for existing units, to start new units and to give proper support to university research.

One unit to be backed up to the hilt is the Laboratory of Molecular Biology at Cambridge. A search committee has been set up to find a director to replace Sydney Brenner, who will be retiring from the post by 1987 and Sir James said that the

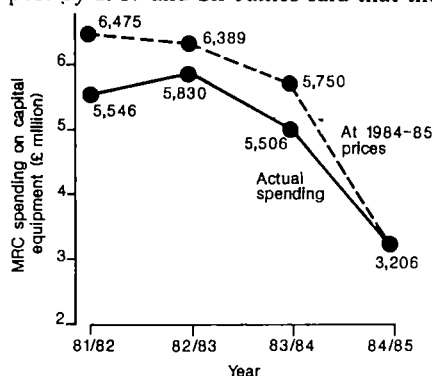
headed by Sir Michael Stoker that is due within the next two weeks.

Although MRC is well off relative to the other British research councils, with a budget that is falling only slightly in real terms, it has such big inescapable costs — mostly permanent scientific staff — that grants to university researchers, spending on capital equipment and the number of research studentships awarded have all fallen markedly in the past two years. For example, capital equipment spending has dropped from £6 million in 1982–83 to £3 million in 1984–85. Of projects worth £4 million rated to be of high priority for which central funds were sought this year, only £2 million could be found, although some more will follow from the extra £2.5 million recently added to the science vote.

Similarly, whereas it was possible in 1982–83 for MRC to support 80 per cent of the alpha-graded grant requests from universities, the figure has now fallen to 55 per cent. Most worrying, says Gowans, is that the total number of applications has fallen from 1,200 to 950 in the same period, suggesting that the scientific community is losing hope.

Unconventional ways of funding have been used for MRC's two current major initiatives. For the Institute of Molecular Medicine in Oxford, both the Wolfson Foundation and the Edward Penley Abraham Research Fund have contributed substantially to capital costs, and the research groups within the institute will be expected to compete for grants rather than be tenured. And the Centre for Collaborative Research, which will occupy a building being vacated by the Imperial Cancer Research Fund next to the National Institute for Medical Research at Mill Hill in London, is expected rapidly to become self-financing as a result of industrial backing.

Peter Newmark



council will have "some flexibility" in the salary that can be offered, although it will not be possible to compete with US universities or industry.

On the other hand, the MRC Pneumoconiosis Unit at Penarth has been closed because the science was not good enough and the Trauma Unit in Manchester is closing because it proved impossible to find a new director of sufficient calibre. Moreover, it is rumoured that two neuroscience units will soon be closed down, while the Clinical Research Centre must be nervous about the report of a committee

Polish universities

Government admits sackings

THE Polish government has now admitted that more than seventy university and higher college rectors, proctors and deans and department heads have lost their administrative posts, almost twice as many as was first reported.

An official announcement last Friday explained that under the amendments to the Higher Education Act, passed last July, the Minister of Science and Higher Education has the right to "review" senior university staff, not only on the basis of their academic work, but also of their "civic attitude". By the end of last month, university officials whose work or "civic attitude" was unsatisfactory had to be informed that they would not be confirmed in the offices to which they had been elected before the change in the law.

The dismissals have been interpreted throughout Poland as a move to regain some of the government and party control over the universities lost during the "liberalization" of the Solidarity era. This is, to some extent, confirmed by the official announcement which stresses that universities must be run according to what it calls the "state and socialist character" of higher education. Throughout Poland, students and academics have been vocal in condemning the changes, and at least one rector not himself affected by the dismissals, Dr Kazimierz Szebiotko of the Poznan Agricultural Academy, is said to have

resigned in sympathy.

Concern has also been expressed elsewhere. In London last week, the Polish ambassador, Mr Stefan Staniszewski, was summoned to the Foreign Office for what was officially described as a "sharp" exchange of views with the Minister of State Malcolm Rifkind. Mr Rifkind warned the ambassador that the dismissals were bound to have an adverse effect on British parliamentary and public opinion, and said that they did not sit well with official Polish assurances that normalization and reconciliation were proceeding satisfactorily in Poland. Mr Staniszewski is said to have expressed surprise that the British government should interest itself in an internal Polish matter, and said that the British government should stick to its 1981 stance that internal developments in Poland were for the Polish people to settle. Mr Rifkind replied that an expression of concern did not constitute interference, but that the dismissals made the Polish government claims seem rather hollow.

Mr Staniszewski himself departed from his stance of non-interference by observing that those concerned in the changes had not lost their teaching jobs whereas in Britain thousands of university lecturers were out of work. In the Polish universities, however, there seems to be a consensus that the dismissal of the officials is only the first step.

Vera Rich

Nobel peace prize

Controversy over the winner

THE 1985 Nobel peace prize ceremony this week was surrounded by last-minute controversy over one of the two persons delegated to receive it, Dr Evgenii Chazov, Deputy Health Minister of the Soviet Union.

The prize was awarded to the organization International Physicians against Nuclear War. In October, when the prize was announced, it was stated that two officials of the organization would represent it at the ceremony in Oslo, Dr Bernard Lown from the United States and Dr Chazov from the Soviet Union. The trouble started, late in November, when it became widely realized that Dr Chazov had, in 1973, signed a letter from Soviet scientists attacking Academician Andrei Sakharov, who himself received the Nobel peace prize in 1975. Some human rights groups called for the 1985 prize to be withdrawn, and one group organized an "alternative peace prize" ceremony in honour of Dr Anatolii Koryagin, now serving a 7-year labour camp sentence for having published in *The Lancet* in 1981 a detailed study of the political misuse of psychiatry in the Soviet Union.

Dr Chazov's co-recipient, Dr Lown, has

been vocal in his defence. Dr Chazov, he says, has said nothing against Sakharov "since 1980". That was the year when the International Physicians against Nuclear War was founded, and also the year in which Dr Sakharov was exiled to Gor'kii. It is not clear which of these events (of both) Dr Lown had in mind. In fact, as recently as last week, Dr Chazov, speaking on the Norwegian ARD-Tagesthemen news programme, said that he was "not in agreement" with some of Sakharov's statements on the nuclear arms race, and quoted an unnamed US physicist to the effect that Sakharov was being made "a prawn in the cold war" and exploited "in a pharisaical manner" in pursuit of anti-communist aims.

Dr Lown, however, seems to have had considerable difficulty with his media statements. In particular, he was reported to have told the wire services that he considered it "shameful" that the US ambassador to Norway had decided not to attend the award ceremony, and then, several hours later, to have told the BBC's "World At One" programme that this was the first he had heard of the ambassador's decision.

Vera Rich

US defence contracting

NASA chief steps aside

Washington

THE US Army's Sergeant York anti-aircraft gun, whose development was cancelled in August after \$1,800 million had been spent on it, seems more effective at damaging people than aircraft. Last week, Mr James H. Beggs, administrator of the National Aeronautics and Space Administration (NASA) for the past four years, was forced to take indefinite leave of absence after being indicted by a Los Angeles grand jury for complicity in an alleged fraud by General Dynamics on the US government.

General Dynamics, best known as the constructor of the *Trident* submarine and the F16 fighter aircraft, has been one of several military contractors in trouble with the Department of Defense over cost accounting, and was suspended from receiving US government contracts for a period earlier in the year. Mr Beggs was a vice-president of General Dynamics, and the one in charge of the company's brief involvement with the anti-aircraft gun until he moved to NASA.

This unexpected development is bound to be a setback for the agency. It seems to be agreed that the year ahead will decide how quickly, if at all, NASA secures the funds with which to build the permanent space station on which NASA has set its heart.

Against the background of a probable budget squeeze early in the new year, this is also a time when an agency cannot easily afford to lose its chief administrator for an extended period.

Both Beggs and General Dynamics hotly deny the charges levelled against them, which centre on allegations that the company, while working on a fixed-price US Army contract to make prototypes of the Sergeant York gun, charged excess costs on the project against other military contracts.

The amount involved is alleged to have been \$7.5 million in a total contract price of \$40 million. On the basis of the prototypes, the development contract was eventually awarded to the other bidder, Ford.

At no point does the Los Angeles indictment suggest that the alleged fraud was other than for the benefit of the company. Three other officials of General Dynamics have been indicted, together with the company itself. Company spokesmen have been suggesting that the origin of the charges lies in the procedures used for charging particular costs to various contracts.

The length of Beggs's absence from NASA is undetermined, but will presumably be at least as long as the time it takes for the case to come to trial.

British pharmaceuticals

Spin-off for research

THE Wellcome Foundation, the British pharmaceutical manufacturer, last week announced its best results ever. This development may have important consequences for British medical research because the company's sole shareholder, the charitable Wellcome Trust, already a substantial source of research funds, plans to sell 20 per cent of its major assets early in the new year. The higher this year's profits, the higher next year's share price is likely to be.

Wellcome's annual report for 1985 says that an increasing share of its business now derives from the United States, and that last year's profit of £121.7 million is largely accounted for by the then favourable exchange rate between the dollar and sterling. Even so, profits are up by 37 per cent over last year, and amount to 12 per cent of the turnover of £1,004 million. Thanks to aggressive marketing, the US business accounted for 45 per cent of turnover and 73 per cent of the profit during 1985. The company spent £122 million on research and development last year.

For the year ahead, the pharmaceutical business hopes to do well with innovations prompted by concern over AIDS (acquired immune deficiency syndrome). Wellcome has to decide soon whether to seek to market its diagnostic tests for AIDS in the United States; the test is one of two selected by the Public Health Service in Britain for the routine screening of all donated blood, which began a few months ago.

Through its US subsidiary, Burroughs-Wellcome, the company is also hoping to make some headway with the antiviral compound azidothymidine, otherwise known as AZT, which is said to inhibit the multiplication of HTLV-III (human T-lymphotropic virus type III) in cell cultures and to have done well in toxicity tests in human subjects. Wellcome says that the drug development process has been shortened from three years to three months in the development of AZT so far, which is a measure of the eagerness of drug houses to put an anti-AIDS drug on the market. But Wellcome is exceedingly cautious about the potential of AZT, no doubt so as to avoid embarrassment of the kind following last month's announcement from Paris of a "successful" treatment of AIDS (see *Nature* 318, 3; 1985).

The sale of 20 per cent of Wellcome's assets in January 1986 is expected to raise £300 million for the trust. The trust will then spend an extra £10 million per year on the support of permanent medical research groups in British universities (see *Nature* 317, 662; 1985), which, added to its present expenditure of £20 million, will make it the single largest supporter of such research in the United Kingdom, spending more on medical research in the uni-

versities than does Britain's Medical Research Council.

The trust is still trying to decide how to attract the best projects. Last week it announced an award to Professor Neil Brooks and Dr James McCulloch at the University of Glasgow for the study of Alzheimer's disease. The research will be

Advanced ceramics

Rush to metal/oxide composites

Boston

A NOVEL method of making ceramic metal composites, known as the lanxide process, was a major attraction at this year's autumn meeting of the Materials Research Society in Boston. The new process, rumoured for some months, was described in public for the first time by Dr Mike Newkirk of the Lanxide Corporation of Newark, Delaware. It promises new tough ceramic composites at significantly lower cost than existing methods, which tend to be expensive and produce a brittle end result.

Lanxides are formed by reaction between a molten metal and a vapour-phase oxidant, for which air will suffice. Typically, the metal has to be doped with a least two dopants — magnesium and silicon work for aluminium — and the temperature of the melt brought to within set limits (1,250°C in this example). The lanxide, in this case a coherent composite of aluminium and interconnected aluminium oxide, forms at the metal surface.

The mechanism of the reaction remains obscure. The material grows from the metal/oxidant interface towards the oxidant, and metal is transported through the growing lanxide by a process that appears not to be reliant on diffusion. The properties of the material, which can be grown in slabs an inch thick can be adjusted by altering the temperature of the melt and by depleting (or not) the reservoir of molten metal.

The microstructure, which reveals a millimetre-scale columnar grain, changes over the cross-section of the lanxide. By appropriate choice of conditions, tensile strength or toughness of an aluminium/aluminium oxide lanxide can be increased significantly above that of sintered alumina. No details of other lanxide materials were given last week, but Newkirk said that "hundreds" of inventions arising from the new technology are being patented.

Military and proprietary interest in the new materials is intense. Obvious applications include rocket and jet engines, armour plating and the Strategic Defense Initiative, where there will certainly be a demand for strong but light materials. According to Professor Rustum Roy of Pennsylvania State University, the im-

portance of the process was brought to the attention of President Reagan soon after it became apparent, and much of the development has been funded by the Defense Advanced Research Projects Agency under conditions of strict secrecy, with arms traffic regulations being invoked to restrict access of technical details to foreigners. Earlier this year, Lanxide Corporation, co-founded by Newkirk two years ago to commercialize the process, received an \$18 million cash injection from Alcan Aluminum Limited in exchange for a 42 per cent holding. Joint ventures between Lanxide and other companies are now also being negotiated.

Maxine Clarke

Tim Beardsley

Bad day for Yonas

THINGS did not go too well last week for Dr Gerold Yonas, chief scientist of the Strategic Defense Initiative (SDI) Organization, when he addressed the Materials Research Society on "Materials for SDI".

Reliability of complex systems has surfaced as a potentially major problem for SDI, and half of the organization's budget is devoted to battle management systems and communications and control. But the frailty of even the most carefully staged events was emphasized when Yonas got up to speak, only to find himself completely inaudible to the audience of close on a thousand. Conference aides fussed and fumbled for a couple of minutes before it was discovered that someone had forgotten to switch on Yonas's microphone. The irony was lost on nobody.

Yonas proceeded to gallop through his usual one-hour presentation on SDI, spiced with some new references to the need for strong and light materials in, for example, space-based reflectors for lasers, and for materials with low coefficients of expansion in infrared lasers. The talk would have been better suited to an audience of high-school students or perhaps congressmen than to a technically-sophisticated group of researchers, and the applause was less than enthusiastic. After some generally hostile questions, Yonas left, perhaps wishing he had not bothered to come.

Tim Beardsley

Information technology

Britain's flagship looks for help

FLAGSHIP, an optimistic collaborative project between the British government, industry and two university departments, was launched in London last week. A £9.3 million investment is being made by the Department of Trade and Industry's Alvey programme with a consortium comprising ICL (Britain's only major computer company), Plessey, Imperial College London and Manchester University, increasing to £15.5 million for the initial three years of the project. Flagship is the long-awaited fifth-generation information processing system with many general-purpose applications. But whether the British project can keep up with Japanese and US efforts is open to question. Certainly legal squabbles over who is to reap the profits from products developed by Flagship have delayed the start of the project.

The general aim of Flagship is to evolve an architecture with sophisticated parallel hardware and "intelligent" software that will be easier for the non-technical user to operate. Imperial College has developed the software for Flagship (the Alice graph-reduction machine) and the hardware is provided by Manchester University's Dataflow machine. ICL is to develop the system at its Manchester site. The Alice prototype uses Inmos transputer chips, but ICL says that recent worries about the future of Inmos will not affect Flagship —

"we already have all the transputers we need".

The Flagship project will concentrate on declarative styles of programming, and will use Dactl, Alvey's compiler target language, which Alvey hopes will be adopted as a European standard. Flagship also uses Pisa (persistent information space architecture) for interfaces between applications, computers and data.

The Alvey programme was set up in 1983 to stimulate research on advanced information technology; its priorities are intelligent knowledge-based systems, man-machine interfaces, software engineering and very large-scale integration. The aims of fifth-generation computers were set out by Japan in 1982; such an architecture should produce computer

systems that work 1,000 times faster and handle 10,000 times more information than existing machines, as well as the "social" function of communication in a natural language rather than machine codes.

The applications of the Flagship type of system are enormous — project management, "knowledge" databases, image processing, robotics and office systems are some areas that Flagship will be seeking to develop in the next few years. The biggest threat to Britain's commercial aspirations is seen to be from the Japanese, who are putting considerable effort into advanced systems architectures. For example, Japan supports eight giant electronic corporations that make microchips, compared with Britain's single troubled company, Inmos. At Flagship's launch last week, Mr Geoffrey Pattie, minister for information technology, hinted that Britain might look for European collaboration for future stages of the project.

Maxine Clarke

European Synchrotron Radiation Facility

Grenoble siting hits a snag

THE Prime Minister of France, M. Laurent Fabius, may have broken the law when he decided to site the European Synchrotron Radiation Facility (ESRF) at Grenoble rather than Strasbourg. That at least is the opinion of M. Jean Raymond, an adviser to the local government of Strasbourg, who is planning to bring the issue to a head this week. M. Fabius has only just emerged, apparently unscathed, from the trouble caused by his remark last week that he had been "troubled" by his president's reception of General Jarulewski at the Elysée Palace.

M. Raymond's case against the Prime Minister was argued at a public meeting in Strasbourg a week ago, and is based on an agreement between the national government and that of the Alsace regional council which was signed in April 1984. The national government apparently agreed to "support the candidature of Strasbourg as a site for new international organizations". It supplied a list of eligible organizations, among which ESRF was one.

One question is whether all the items on the list were to be considered for Strasbourg in the first instance, or whether they were merely examples; Raymond believes the intention was clearly the former. But the second more substantial question is whether the agreement can be legally binding whatever interpretation is correct. Raymond argues that planning laws enacted in 1982 and designed to devolve power to the French regions do indeed have the force of law. He seems to have a point when he adds that none of the procedures specified in the 1982 act for rescinding agreements between central and local governments seems to have been followed when ESRF was moved to Grenoble.

A dispute between the regional government, which belongs to the political right,

and the socialist government in Paris thus seems inevitable. M. Fabius's case would undoubtedly be stronger if the machine intended for Grenoble were already partly built. But as yet, no ground has been broken there; indeed, the government is still seeking foreign contributions towards the project. So the return of ESRF to Strasbourg is not impossible.

Robert Walgate

Satellite insurance

ARIANESPACE, the French-based company that sells space on the European space launcher Ariane, has moved quickly to offer customers insurance at reasonable cost (see *Nature* 317, 567; 1985). The company has set up a subsidiary, the Société de Réassurance des Risques Spatiales (known as S3R), which will offer users a free re-launch of a failed satellite.

S3R is designed to cover the next fifteen Ariane launches over the next five years, and will offer insurance at premiums of 10–13 per cent, compared with the 20–30 per cent being quoted unofficially by the open markets. The difference stems from S3R's sanguine view of the risks. It expects only one failure in the next fifteen Ariane launches, compared with the one-in-five risk expected by private insurers.

Even so, the new company expects to lay off some of its risks on the private re-insurance market. S3R is meant only to cover the next three years, after which it is hoped the insurance market will have been reassured by Ariane's performance.

Whether the terms now offered constitute a subsidy will be fiercely argued both by the private insurers and by those who regard Ariane as a serious competitor of the space shuttle.

Robert Walgate

UNESCO minus one

THE British government last week formally announced its resignation from UNESCO (United Nations Educational, Scientific and Cultural Organization), to the consternation of many remaining members. The £6.4 million that Britain contributed will be transferred to the British Council, Mr Timothy Raison, minister for overseas development, said last week. The government will use the money to enable more students from the developing countries to study in Britain; numbers are expected to increase from 11,000 to 12,000.

Members of other European Community countries, particularly France and West Germany, Japan and members of parliament from the three major parties in Britain, all objected strongly to Britain's decision to withdraw. Mr Amadou Mahtar M'Bow, UNESCO's director general, expressed his "deep regret" at Britain's departure.

Britain, after the departure from UNESCO by the United States last year, had demanded many reforms in the organization (see *Nature* 14 November, p.98; 5 December, p.397). Some were implemented at the recent general assembly in Sofia, but Britain felt that UNESCO had not gone far enough.

Maxine Clarke

US bomb tests

Radiation tests underestimated?

Washington

THE US Department of Defense (DoD) was criticized last week by the General Accounting Office (GAO) for underestimating the exposure to radiation of 42,000 service veterans who participated in the 1946 "Operation Crossroads" atomic weapons tests at Bikini Atoll. According to GAO, the DoD's assessment, on which basis the Veterans Administration has so far rejected medical claims based on radiation exposure, may underestimate or entirely overlook significant beta exposure, while the internal alpha dose may be underestimated by a factor of 5 to 10.

The two Crossroads explosions, the first above water and the second below, were the first atmospheric weapons tests in peacetime, and radiological protection procedures were crude or non-existent. Decontamination procedures, showering and changing of clothes, for men working to clean up ships moored near the explosion, were only instituted six days after the second (and much dirtier) test, as the extent of contamination became apparent. The clean-up was eventually halted because of high radioactivity level, but only 6,300 of the 42,000 participants in Crossroads wore film badge dosimeters, and none wore badges every day.

Because records are so incomplete, DoD in 1977 asked its Defense Nuclear Agency to reconstruct exposures by extrapolation from known cases. The agency's conclusion, published last year, was that 93 per cent of those involved received exposures of less than 0.5 rem, and that all received less than 1.7 rem (the permitted total for radiation workers in the United States today is 5 rem per year). But GAO has identified several respects in which it believes DoD's analysis is in error.

Most doubt centres on the accuracy and interpretation of film badges. The badges did not work in the way expected, possibly

because of the effect of humidity, and GAO believes beta exposures could as a result have been seriously underestimated: 100 per cent error can be expected at the lower end of the range, for which DoD made no allowance. GAO also finds that personnel decontamination and protection procedures assumed by DoD were not in effect (immediately after the tests) or were widely ignored; in none of the photographs of the clean-up operation are the men wearing protective clothing.

DoD has formally disagreed with many of GAO's conclusion, but GAO retorts that DoD's comments are based on incor-

rect and unsupported statements and misinterpreted documents. GAO's recommendation is that DoD be required to revise its estimates to take account of the sources of error now identified. If the revisions are carried out and GAO's conclusions validated, the implications could be great for the 500 veterans' disability claims relating to Crossroads, as well as to the many thousands of others relating to different tests; the Veterans Administration has already indicated that it wants to extend GAO's findings. The GAO report, which was requested by Senator Alan Cranston of the Senate Veterans' Affairs Committee, will doubtless form the background to what may be expected to be stormy congressional hearings in the near future.

Tim Beardsley

Computers

Putting back the clock

Berkeley, California

CONVENTIONS held sacred for decades by the designers of computer hardware and software are being seriously challenged by computer researchers at the University of California, Berkeley. At the fore of the challenge is a new development in microchip technology which has simplified the design of the electronics needed for computers and jettisoned redundant and laborious machine coding.

That part of the Berkeley revolution began about five years ago, but its proponents believe the change is at last about to be accepted in the industry despite the controversy the ideas have generated and the hostility they have provoked. According to David Patterson, an associate professor of the Computer Science Division at Berkeley, and one of the creators of the new machines, "1986 will be the year when we see what happens".

The new computer designs were arrived at by inspecting a VAX minicomputer and identifying many machine code instructions that were never used in most com-

putational exercises. In that inspection, 304 such instructions were reduced to one tenth that number. So was born the Berkeley computer concept, called Reduced Instruction Set Computers (RISC).

The RISC machines turned the clock back in computer design. The evolution of computers — mainframe, mini and the micro designs in the 1970s and 1980s — used volumes of complicated instructions (the basic code programming the machines) and relied more and more on intricate and faster microelectronics to control that complex structure. A computer evolved within the computer, controlling these arrays of instruction codes, most of which were never used.

Berkeley's idea was to minimize the coded instructions needed to operate the machine and, at the same time, to design a simpler microchip. The RISC microchip made at Berkeley, the hub of the new machine, now has over 41,000 transistors and 60 32-bit registers. It can be fabricated on silicon down to a gate size of 4 μm with a speed of 500 ns per instruction.

Berkeley's research has been matched by that of others at Stanford University, IBM at Yorktown Heights and now at Hewlett Packard. Says Patterson, "Hewlett Packard is going to base its whole next generation on these ideas". IBM is rumoured to be developing workstations on the same principles.

Patterson is confident that RISC is a forward step despite its apparent resort to simpler technology. "Many people find this to be a ridiculously bad idea. It's a de-evolution of computer design... a dangerous concept. Shouldn't be talked about". Patterson and his Berkeley team are continuing to talk about it, and are developing the concept even further to see if the RISC machines can be further enhanced for the scientific and academic use of computers. One such application to be pursued is the use of RISC for artificial intelligence.

Bill Johnstone

Soviets admit AIDS cases

THE Soviet Union has now admitted that AIDS (acquired immune deficiency syndrome) has reached the Soviet Union. Interviewed in the newspaper *Sovetskaya Kultura*, Dr Viktor Zhandrov, a leading Soviet virologist, denied the growing rumours that there are "numerous" cases of AIDS in the Soviet Union but acknowledged that there had been an "insignificant number of cases" reported.

This comes less than two months after Soviet denials that there were any cases of AIDS in the Soviet Union (see *Nature* 317, 659; 1985).

Dr Zhandrov's article is less polemic in tone than previous Soviet statements on the disease, which stressed its connection in the West with drug addiction (relatively

rare in the Soviet Union) and homosexuality (which is illegal).

He stressed, moreover, that the disease seems to have originated in central African monkeys. This is in marked contrast to a recent trend in the Soviet media to attribute AIDS to United States bacteriological warfare experiments; although much of this reporting seems to be anecdotal (for example, that in Kenya AIDS has developed in proximity to a US base), not all of it has been directed at Third World audiences. In particular, on 30 October, *Literaturnaya Gazeta* quoted the Indian popular daily, *The Patriot*, to the effect that AIDS was developed in the United States at Fort Detrick as a weapon against the black and homosexual communities. Vera Rich

Japanese research

Private investment still growing

Tokyo

RESEARCH in Japan's private sector is booming as never before. Expenditure has grown 12 per cent this year (eight times as fast as the government science budget) with 78 per cent of the nation's research funds now coming from industry. These figures set the scene for the Science and Technology Agency (STA)'s white paper, *New developments in R & D and the age of cooperation*, which was approved by the cabinet last Friday.

The aim of the white paper is not so much to sing the praises of industry as to point out some weaknesses that in part stem from such a high reliance on the private sector and to suggest ways of correcting them. One new bill aimed at increasing cooperative research is all ready to go before the Diet.

Overall investment in research and development is growing proportionately faster than the economy and has now reached 2.8 per cent of national income, not far short of the 3 per cent goal set by the government. Virtually all companies involved in high-technology industries — electronics, telecommunications and biotechnology — are spending at least 8 per cent of their sales on research and development.

The other side of the coin is the relative weakness of government and university research and failure of budgets to keep up with inflation for several years running. This means that there are weaknesses in areas that industry cannot easily tackle; those requiring large-scale interdisciplinary cooperation and those requiring long-term cooperation for an uncertain pay-off.

Lack of government impetus partly reflects low government expenditure, and partly low levels of collaboration between the universities, government research institutes and industry. This is a perennial problem, but the need to get something done seems more urgent than before. Almost all advanced nations are pushing hard in the same high-technology fields. And as the report neatly points out, they are all adopting very similar science policies; everywhere industrial investment is being increased through similar packages of tax incentives and policy designed to spur cooperation between different research fields.

The white paper identifies key problems and, not surprisingly given that the paper is the work of a government agency, suggests solutions that nearly all involve increased expenditure at government laboratories. Two of the problems are interrelated; *per capita* expenditure on basic research is still low compared with Western nations, and basic research in public laboratories is insufficiently creative. The first is a problem of money. Unfortunately whatever STA might believe is correct,

there is little chance that the government will increase basic research funding when it has a fiscal deficit proportionately larger than that of the United States. The second is more a problem of organization and in particular failure to provide young researchers with the freedom to put their talents properly to work. The problem is now to be taken up by the Council for Science and Technology — Japan's highest advisory body chaired by the Prime Minister. A few days ago, the council agreed to a wide-ranging debate on the operational management of the public research institutes.

A third problem seems to stem from a feeling that anything the West is doing Japan should be doing too, in this case its giant research projects such as the Strategic Defense Initiative (SDI) and the emerging Eureka programme which might produce a quantum jump in technological level. There is nothing really new on the horizon, to follow large-scale projects such as the development of the

"bullet" train and automatic telephone switching gear. STA officials suggested that international cooperative projects in cancer research, fusion energy, giant accelerators and space platforms might all be candidates for the development of completely new technologies. But there is little prospect of a major publicly-funded Japanese research initiative under current budgetary constraints.

Positive action is at hand to deal with the fourth problem, that of encouraging cooperative research between industry, government and the universities. The need for some form of cooperative research is obvious to industry: in the past few years, there has been a striking rise in joint research performed abroad. Growth in tie-ups with domestic institutions has been much more sluggish. A new bill to be introduced by the cabinet in March and intended to clear the Diet by the summer should remove at least the legal restraints on cooperation between the public and the private sectors. The bill will also make it easier to carry out cooperative research with foreign companies and tackle problems of patent rights. **Alun Anderson**

CEGB

Acid rain storm from Norway

"UNBALANCED and biased", Norwegian Ministry of the Environment official Jan Thompson said of the British Central Electricity Generating Board (CEGB) video "Acid Rain". The Norwegians pulled few punches at the press conference they had called in London on Monday of this week to give their response to the recently released film. Thompson continued "the views put forward in the CEGB film are obsolete and dangerous . . . and will in the long run strain the relationship between the two countries. A cynical attitude was expressed".

The 20-minute video, widely distributed throughout the United Kingdom, purports to present a balanced and objective view of the acid rain issue, and concludes that the effects on the environment of acid precipitation may have been overestimated and that there is considerable doubt in the scientific community that the widely reported deaths of fish and forests in Europe are caused by acid rain. In addition to CEGB personnel, the film uses interviews with Norwegian, Swedish and German scientists, and was partly shot in Norway. The Norwegians say that neither their environmental authorities nor major research institutes were notified. They also claim that there was considerable bias in the selection of persons interviewed, and that interviews were cut so as to present a distorted view of opinions expressed. At least one scientist, Swede Hans Hultberg, is understood to have protested strongly against the editing of his comments.

The seriousness with which the Norwe-

gians are treating the affair can be judged from the fact that in addition to a report on the video sent to CEGB, a letter from the Norwegian Prime Minister, Kaare Willoch, has been delivered to Mrs Margaret Thatcher. The subject of acid rain was prominent in a meeting between the two late last month. In carefully chosen words, Jan Thompson said "we are not talking about a diplomatic protest".

The Norwegian scientists at the conference — Professor Seip and Dr Muniz from the University of Oslo and Mr Dovland from the Norwegian Institute for Air Research — presented detailed evidence, some new but much previously published, to support their case that some 15–30 per cent of the sulphur deposition in southern Norway originates in the United Kingdom. They argue that the recent reductions in emissions from Britain result only in fine tuning when used in long-range pollutant transport models. The claim is not that stopping emissions from Britain alone would solve the problem, but that concerted European action is needed to stop this "chemical war", as Muniz described it.

The Norwegians claimed that the natural environment is being seriously damaged and that it is disturbing that acid precipitation can be so lightly dismissed by CEGB on the grounds, among others, that 100 per cent scientific certainty has not yet been achieved. Borrowing a symbol for the *Bulletin of the Atomic Scientists*, Seip showed an acid rain clock; the hands stood at five minutes to midnight.

Peter Gambles

Dioxin exposure at Monsanto

SIR—Hay and Silbergeld urge a re-examination of the information about workers exposed to dioxin at the Monsanto plant in Nitro, West Virginia¹. They do not actually challenge the conclusions of two studies that there were no excesses of cancer or heart disease deaths among dioxin-exposed workers, but they convey the impression that the studies are seriously flawed.

In particular, they draw attention to the fact that four men classified as exposed to dioxin in one paper were classified as not exposed in the other. The first paper, which classified the men as exposed, defined exposure by the presence or history of chloracne², a disease known to be associated with dioxin. The other, which classified the men as not exposed, defined exposure by a history of work assignments in processes involving dioxin³. Neither measure is perfect, and they are not congruent. Although the differing classifications are disquieting, at least three of the cancer deaths are probably independent of dioxin exposure. Those three were lung cancer deaths among smokers; the fourth was from Hodgkin's disease, for which there is no strong evidence for association with dioxin. If the differing classifications for the four men are enough to destroy the conclusions reached by the authors of the studies, that analysis should be developed by Hay and Silbergeld.

From Monsanto records that they do not further identify, Hay and Silbergeld state that 19 workers who would be classified as exposed and who died from circulatory disease or cancer were not included in the second mortality study. They do not provide information about how they determined the men's exposure, the number of unexposed men who died from the same causes during the same time period, nor when the men died. If they died before 1955, they would not have been included in the study, which, because of records limitations, was restricted to men who left the company after that time. A telephone call to the authors might have clarified the reason that those men were not included. Whatever the outcome of such a call, it would not have contributed to the aura of conspiracy achieved by the sentence "No reason is given for not including those cases".

Workers exposed as a result of the 1949 explosion at Nitro are generally considered to have been more heavily exposed than workers involved in usual processes at the plant. Hay and Silbergeld, referring to a 1953 study⁴, contend that the basis for that conclusion was more severe chloracne among the accident-exposed workers. While chloracne was worse among those workers, a panoply of other health complaints — fatigue, incapacitating aches and pains, libido and potency problems — was also elevated, lending cre-

dence to the contention that accident-exposed workers were more heavily exposed. I am uncertain what point Hay and Silbergeld want to make as they labour over the conjecture that there may have been no differences in exposure levels, but they follow that effort with criticism of the authors of the mortality studies for separating the two groups of men in their studies. The criticism is misplaced. While one paper² was limited to workers involved in the aftermath of the 1949 accident, the other¹ included all hourly workers who terminated their employment after 1955, whether or not involved in the 1949 accident.

Having found no evidence of excess cancer in the Nitro workers, Hay and Silbergeld turn the idea of dose-response relationships on its head and suggest that diseases such as cancer "may be expected" in people exposed to "lower and more chronic exposures". References offered in support of that idea are two books that contain data both suggesting and not suggesting a link between dioxin and human cancer and two papers associating cancer in herbicide sprayers with dioxin. The herbicide sprayers characterized as chronically exposed were exposed only during one or two months a year⁵, for a median total exposure time of 42 days. Studies of another population, which included more than 1,500 sprayers who had been exposed for at least 12 months, found no cancer excess⁶. Furthermore, some Nitro employees worked for years in production processes that involved dioxin and some, not exposed through the explosion, developed chloracne⁷. Those men were surely chronically exposed to dioxin, many were included in the Zack and Gaffey¹ study, and there is no cancer excess. The hypothesis that low-level, intermittent exposures bear more risk than higher-level exposures requires substantiation.

Hay and Silbergeld's penultimate paragraph refers to a health survey⁸ as support of a possible connection between dioxin and an "excess of cardiovascular-related deaths". The health survey presents no new data about mortality; it does refer to an excess of cardiovascular deaths in Monsanto workers already reported by Zack and Gaffey¹. An editorial that accompanied publication of the health survey suggested that the health survey might include some evidence for an excess of myocardial infarctions (not deaths) among men who had had chloracne⁷, but that the appropriate analysis is wanting. Finally, the mortality study that reported the excess of cardiovascular-related deaths in Monsanto workers found no difference in death rates from the cause between workers exposed and not exposed to dioxin¹, and the number of cardiovascular deaths among chloracne-stricken workers was less than 75 per cent of the

expected number⁸.

Hay and Silbergeld criticize studies that use chloracne as the criterion of exposure. They are on firm ground when they say that the disease is a dependent variable of exposure and that epidemiological studies usually use some independent measure, such as work histories, as the criterion for exposure. However, they raise no criticism of the health survey⁸ for depending on chloracne as a measure of exposure. Apparently, a double standard is used, studies that cannot be interpreted to show excess deaths being held to a higher one.

In Hay and Silbergeld's words, "the epidemiological picture at Monsanto remains confused". The picture is more unclear than anyone would desire, but it is not quite so confused as they have it. Their letter shows that it is possible to locate flaws and select studies to support an argument that dioxin is possibly a scourge. Equally, it is possible to read the literature and arrive at a cautious conclusion that dioxin has not caused early death and cancer in heavily exposed workers. That conclusion, which is widely accepted⁸ and draws on information from dozens of studies done in the past decade, might lift some anxiety from people who have been environmentally exposed to lower levels of dioxin.

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AIDS

SIR—Misinformation on acquired immune deficiency syndrome (AIDS) continues to abound and Ian Davidson's comment (*Nature* 317, 666; 1985) is another example.

It is wrong on two counts: (1) It is perfectly possible to be promiscuous and have little risk of contracting AIDS, providing one avoids certain high-risk sexual practices; (2) Non-promiscuity does not guarantee that one will not contract AIDS. Certain groups are still at risk, for example wives of gay or bisexual men, and intravenous drug abusers.

I do not know if chastity has replaced death as the "great unmentionable", but fiction seems to have replaced fact when it comes to the truth about AIDS.

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Whose rings around Neptune?

What seems to be an authentic and interesting discovery of an incomplete ring around the planet Neptune seems certain to be published piecemeal for lack of agreement among those concerned.

READERS will be puzzled by the tentative explanation of the partial ring around Neptune provided on page 544 of this issue by Dr J. Lissauer of the University of California, Berkeley, chiefly because they have no reason to believe that there is such a structure around this distant planet. Surely, readers will ask, it will be time enough for Dr Lissauer to produce his explanation when there is evidence to suggest the need for one. And why should this apparently pointless explanation begin with the flat unsubstantiated declaration that "an incomplete arc system has recently been discovered around Neptune"?

Dr Lissauer should not be judged too harshly. Evidence for a partial ring system around Neptune does indeed exist. Roughly six months ago, an article giving a full and interesting account of the discovery was submitted to *Nature* for publication, was accepted for publication and would long since have been published had not those responsible for the collection of the evidence fallen out over the wording of the paper. All the authors have throughout expressed distress at being caught up in such a childish business yet have been unable to reconcile their differences.

What follows is an account of the circumstances leading to this sad state of affairs, from which two lessons may be learned. First, there may be occasions when pride takes precedence over the principle that science is an open process, in which publication is a duty owed to the rest of the community. Second, there are some outside the community who will ask whether science can be as deserving of public support as journals such as this repeatedly insist.

One of the prime movers in the discovery of the ring around Neptune appears to have been Professor André Brahic, from the University of Paris and the Observatoire de Paris, who has a long-standing interest in planetary ring systems. Brahic was the first author (among eight) of a manuscript "Occultation detection of a neptunian ring-like arc" which reached the *Nature* office at the end of May this year. The other authors quoted were W.B. Hubbard and F. Vilas from the University of Arizona, who have access to the Inter-American Observatory at Cerro Tololo in Chile, together with L.R. Elicer, a member of the observatory's staff; two colleagues of Brahic's from Paris, B. Sicardy and F. Roques; Patrice Bouchet, a staff member at the European Southern

Observatory (ESO), also in Chile, and two European astronomers, Jean Manfroid from the University of Liège and R. Haefner from the Max Planck Institute of Astrophysics at Munich.

The origins of the dispute among the authors can be traced to the night of 22 July 1984, when there was a near-occultation of Neptune of a bright red star in Sagittarius, then visible from the two Chilean observatories. Brahic and his colleagues in Paris, who seem long to have suspected that Neptune may have a ring system, seem to have taken the initiative in drawing attention to this event. Ironically, Brahic had applied for observing time at ESO on the night in question, but had been given time to look at the rings of Uranus instead. Robbed of this chance, and with the good offices of Bouchet, Brahic seems at second-hand to have persuaded Haefner and Manfroid, the intended occupants of two telescopes at ESO, to turn away from their planned project to look at the occultation by Neptune instead.

In the event, Haefner and Manfroid found that there was indeed a brief second-long interruption of the infrared radiation from the distant star as it crossed an invisible circular orbit roughly 77,000 km nearly 3.0 radii from the centre of Neptune. One curious feature of the observations was that there was no corresponding dip in the radiation intensity as the distant star crossed the same circular orbit at its supposed second interception of it, whence the notion that, if there is a ring around Neptune, it is incomplete.

A similar sequence of events was found at Cerro Tololo, 100 km away in the Andes. Elicer began his observations later in the night, just a few minutes before the expected near-occultation and in some haste, to judge from the circumstance that it is not known what filter had been interposed in one of the two infrared channels. But the results are decisive and essentially the same as those found at the ESO site. Again there was a single interruption of the radiation, lasting about a second but no corresponding dip at the supposed second interception.

It is common-ground that these observations can only mean that there is absorbing material in one part of a supposedly circular orbit about Neptune, but that the material does not extend uniformly around the orbit. Brahic has rightly all along insisted that only coordinated

observations at different sites can lend credibility to such fleeting events. (Clouds, electrical disturbances or passing flocks of birds could be held responsible for a brief interruption of radiation at a single site.) But with a well coordinated pair of observations in hand, Brahic was able to go back to previous attempts to find occulting material around Neptune. The pair of Andean observations last July, from sites separated by 100 km, show that the absorbing material around Neptune must be at least 100 km in extent. The time for which radiation was interrupted suggests an object 15 km across.

So, now there is an interesting and quite novel phenomenon to be explained, a partial planetary ring. If it had been possible to publish the data on which the inference is based, people other than Lissauer would have been stimulated to bend their energies to this interesting problem. But why are the data still under lock and key?

Several things have gone wrong. The ESO observers, Haefner and Manfroid, have been offended by what they consider to have been unfair publicity overwhelming their contributions, a press release from the University of Arizona relayed in semi-popular journals such as *Sky and Telescope*, for example. An article by Brahic in *La Recherche* last June seems to have given particular offence, although all the intended authors of the original *Nature* manuscript are duly listed in the margin. Haefner and Manfroid have published their data in the German-language journal *Stern und Weltraum*.

Much of the difficulty turns on the question of who "owns" the data collected at ESO, the observers who collected it or Brahic who drew attention to the occultation and to whom the data tapes were sent by prior arrangement? And what is to be done with data which, as in this case, are more interesting when combined with other people's than when published on their own?

The saddest feature of this development is that, whoever owns some of the data, there is a sense in which they belong to the scientific community as a whole. The upshot is that an important and even urgent set of data will not now be published quickly, as it should have been. The reputation of astronomers for good sense will have been undermined. Even the reputation of science as a whole will have been damaged — let us hope not irreparably.

John Maddox

Forensic science

DNA fingerprinting in matters of family and crime

from Barbara E. Dodd

IT HAS long been the ambition of the forensic scientist to be able to identify the origin of blood and body-fluid stains with the same degree of certainty as fingerprints. And the ability to settle cases of doubtful paternity with absolute certainty in every case would be equally welcome. The arrival of DNA fingerprinting — particularly as developed by Alec Jeffreys and colleagues at Leicester University^{1,2}, whose latest paper is on page 577 of this issue³ — is a big step towards the forensic scientist's goal in both areas.

As so often happens, the technique has arisen from research that was not aimed specifically at solving practical problems. Jeffreys had been working on the short 'minisatellite' sequence of DNA in an intron of the human myoglobin gene. Using the now familiar methods of Southern blotting and DNA hybridization he found that the myoglobin minisatellite detected other human minisatellites, some of which are highly polymorphic. The common features of these minisatellites have been identified by sequencing, and cloned DNA probes, based on tandem repeats of the core sequences, have been used to detect simultaneously several highly variable genetic loci in the human genome. It is this extreme variability in the pattern of minisatellites detected by the probes,

together with the stable inheritance in the usual mendelian manner of an individual's pattern, that is the basis of DNA fingerprinting.

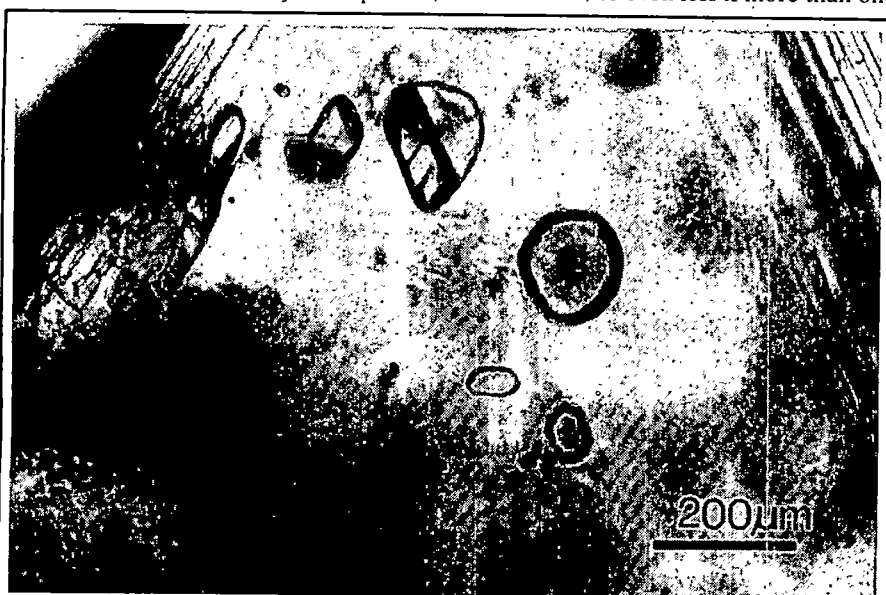
In this issue of *Nature*, Jeffreys with P. Gill and D.J. Werrett of the Home Office Forensic Science Service report promising progress in applying DNA fingerprinting to blood stains, semen stains and hair roots, showing that DNA fingerprints obtained from these sources are always identical to those of the individuals from whom the material originated. Especially rewarding are the results of DNA fingerprints of sperm nuclei and their potential for the identification of rapists. There are conventional genetic markers in semen and semen stains are frequently contaminated with vaginal fluid, so that the origin of any markers present is difficult or well-nigh impossible to trace. Gill *et al.* have managed to isolate sperm nuclei free from vaginal contamination and have obtained a DNA fingerprint that is the same as the donor of the sperm. Thus it should be possible to match a DNA fingerprint of sperm from, for example, a stain on a victim's clothing with that of blood or sperm from a suspect rapist. A chance match is virtually eliminated because its probability is calculated to be of the order of 3×10^{-11} , or even less if more than one

probe is used. The technique, however, has yet to be tested and assessed for application in actual casework. How easily DNA fingerprinting can be assimilated in a routine crime laboratory remains to be established.

Other work is in progress on the feasibility of using DNA polymorphisms in the examination of blood and semen stains. M. Baird and colleagues of Lifecode Corp., New York reported at the 11th International Congress of the Society for Forensic Haematogenetics in Copenhagen last August. They have used probes that recognize two highly polymorphic DNA sequences to show that DNA recovered from blood stains up to three years old can be used for identification purposes and that the DNA fingerprints of sperm recovered from female volunteers after sexual activity, when freed of female cells, is identical to that of the male partner's sperm.

The numerous blood-group polymorphisms that are currently applied to cases of doubtful parentage (usually doubtful paternity) already go far towards excluding every man wrongly named as father of a child. Exclusion of paternity is established by showing the absence in the putative father of one or more blood-group gene products, which, in the child, are seen to be paternally inherited. A range of polymorphic systems are investigated independently of each other by relatively simple procedures in which the gene products are disclosed by agglutination, cytotoxicity tests or some form of electrophoresis. The table shows how a combination of polymorphisms can approach the ability to exclude all false fathers. Moreover, the calculated probability of paternity for unexcluded men, virtually all of whom will be true fathers, is likely to be significantly in favour of their paternity.

Even so, DNA fingerprinting with the probes developed by Jeffreys has the potential to exceed the efficiency of the conventional systems because there are good grounds for anticipating that definitive answers, not only those excluding paternity but also for pinpointing the true father, will be obtained in every case. But it is in resolving problems of family relationships in which putative parents are closely related to each other that DNA fingerprinting has particular advantages over presently used systems. This has already been demonstrated by Jeffreys *et al.*³; the problem was that of a Ghanaian boy who was refused entry into Britain because the authorities were not satisfied that the woman claiming him as her son was in fact his mother. Analysis of a range of systems similar to those in the table showed that the alleged mother and son were almost certainly related (probability of non-relationship 0.01) but the conventional tests were not capable of determining whether the woman was the boy's mother or aunt. DNA fingerprinting established that the mother-son rel-



Unique garnets in diamonds

Enlarged view of a diamond, from the Monastery Mine kimberlite pipe in South Africa, that contains two garnet inclusions (on the right-hand side, with dark shadows surrounding them) and four jadeitic clinopyroxene inclusions. The garnets are the first of natural occurrence to contain pyroxene in solid solution, which indicates an unusually deep origin for the diamonds. This discovery and its implications for mantle structure are described by R.O. Moore and J.J. Gurney on page 553 of this issue.

ationship was correct because the minisatellites detected by the probes are so hypervariable that the chance of a sister of the alleged mother sharing all the maternal specific bands of the child was 6×10^{-6} .

DNA polymorphisms have also been investigated with respect to US paternity problems, as reported by I. Balazs at the Copenhagen congress. He and his colleagues at Lifecode Corp. have examined DNA polymorphism associated with two genetic loci in more than 100 paternity cases and have shown that their probes produce an exclusion probability similar to HLA (see table). So far their results have not been presented in court. The American Association of Blood Banks committee on parentage testing has studied the implications of paternity testing by DNA and has issued guidelines. These include the need to validate DNA probes by extensive family and population testing before they are used in parentage testing; the estimation of gene frequency and mutation rate for the genes being detected; the inclusion of duplicate gels and blots; and, at least for the present, parallel testing with accepted systems.

Although DNA fingerprinting as developed by the Jeffreys team requires but a single series of manipulations to disclose a richer variety of polymorphisms than shown in the table, the process is very labour-intensive and needs both meticulous expertise and much experience in the reading and interpretation of the bands that appear in the final autoradiographs. And since the outcome in human terms is likely to be far reaching and since no confirmatory tests using other independent systems are possible, it is surely essential to duplicate each test. A two-tier procedure may develop, beginning with a range of conventional systems and proceeding to DNA fingerprinting in selected cases.

One can foresee that through the energy of molecular biologists, probes suit-

Chance of exclusion of non-fathers	
System	Chance (%)
Red cell antigens	
MNSs	32.1
Rh	28.0
Kidd	19.0
Duffy	18.0
ABO	17.6
Kell	3.3
Lutheran	3.3
Serum proteins	
Gc	24.7
Hp	17.5
Glm	6.5
Km	6.0
Red cell enzymes	
Phosphoglucosmutase	25.3
Erythrocyte acid phosphatase	21.0
Glutamate-pyruvate transaminase	19.0
Glyoxalase	18.4
Esterase D	9.0
Adenylate kinase	4.5
Adenosine deaminase	4.5
HLA	94.0
Total for all systems combined	99.7

able for appropriation by forensic scientists will proliferate while techniques for DNA fingerprinting may become simpler. Excitement concerning the application of DNA fingerprinting to forensic science has to be tempered by the realization that much assessment remains to be done. If this proceeds satisfactorily it will be difficult to disagree with the prediction of Gill *et al.* that DNA fingerprinting will revolutionize forensic biology. □

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mass of the asteroid zone would have remained reasonably constant throughout most of the life of the Solar System, the main loss having occurred as a result of the primordial process that stirred up the zone — possibly perturbations by a proto-Jupiter. This stirring changed the low interparticle velocities at which accretion could take place into much higher impact velocities which resulted simply in collisional fragmentation.

One way of discriminating between these two possibilities is to look at the collisional evolution of the present asteroid belt and to wind this back to see if one gets a reasonable initial population or not. If the population is large the first process is sufficient. The second process would indicate that the asteroid zone has had a low mass ever since the growth of proto-Jupiter. This has significant implications for the degree to which asteroidal spins have been modified by collisions and for the amount of fragmentation that asteroids have suffered.

Consider the observational constraints. Davis *et al.*³ note that Vesta (the fourth largest asteroid, diameter 480 km) has a largely intact basaltic crust, indicating that it has not been shattered during the pre-primordial period and that the number of bodies capable of breaking up Vesta must be low. A significant number of asteroids are members of families having similar orbits and having been produced by the disruption of a single parent, generally thought to have a diameter about 200 to 300 km^{4,5}. The fact that the families persist indicates that collisions are rare.

A third, less strict, constraint comes from collision modelling³. The frequency of collisions at 5 km s⁻¹ between asteroids of, say, 100-km and 500-km diameter is quite well known but the outcome of the collisions is not. In laboratory studies, collision energies reach around 10¹ ergs, which is less than 10⁻²¹ of the kinetic energy of a body with a 100-km diameter.

Farinella *et al.*² turn to the rotational properties of asteroids as the fourth constraint. About one third of the asteroids in the 200–300-km-diameter range seem to have elongated shape, indicated by large light-curve amplitudes and rapid spins. If this was induced by collisions there must have been about five times as many asteroids larger than 65 km in the initial (post-primordial) population than there are now. A similar conclusion is reached by Davis and colleagues³.

The history of the asteroid zone is still somewhat vague. Greenberg *et al.*⁶ found that it would take about 10⁵–10⁶ years to accrete Ceres if there was initially a total rock mass of about the mass of the Earth, all in the form of 1-km bodies. Then something must intervene to terminate the accretion. Maybe it was orbital stirring by proto-Jupiter. If so, Jupiter has to accrete very quickly, more quickly than the asteroids, and one is still left with the problem that the stirring process itself takes about

Solar System

Missing mass in the asteroid zone

from David W. Hughes

LIKE the Cassini division in Saturn's rings, there is a division in the Solar System, at the asteroid zone. Both these divisions indicate regions of high mass depletion. At present, the asteroid belt between the orbits of Mars and Jupiter contains an observed¹ mass of about 5×10^{24} g, about one thousandth the mass of the Earth. If the distribution of mass in the proto-planetary solar nebula were reasonably uniform there would have originally been sufficient rocky material in that zone to form a planet or planetary core of at least the same mass as the Earth. What has happened to this mass? Farinella, Paolicchi & Zappalà² suggest two alternatives —

either there has been continued gradual loss of mass or the main mass was lost primordially and little has been lost since.

The gradual process would result from asteroids breaking up as they collide together at a mean speed of around 5 km s⁻¹. Most of the debris has speeds well above the escape velocities of the two colliding asteroids and so move off into the zone. The Poynting–Robertson effect, that is the symmetrical re-radiation of absorbed unidirectional solar radiation, retards the dust, causing it to spiral in towards the Sun. Over the age of the Solar System this has depleted the asteroid population. In the second process, the

10^6 years. The orchestration of this process has to be finely tuned. If Jupiter formed too early, premature stirring would have prevented the accretion of large Ceres-type asteroids. If Jupiter formed too late, accretion in the asteroid belt would have continued for too long and there would now be a planet there. It was probably close run — if Ceres were about twice its present size, it would accrete even at impact velocities of 5 km s^{-1} .

Another suggestion⁷ is that accretion stopped and the asteroid velocities were increased by secular resonances sweeping through the zone. These were caused by mass loss from the Sun, this mass being dissipated in the solar nebula during the Sun's T-Tauri phase. Unfortunately, for this to work, the T-Tauri phase would have to be exceedingly active and to have lasted for about 10^4 years.

Whichever velocity-increasing process we choose, it still has to explain how the original mass of the zone (one Earth mass) was reduced to the post-primordial mass, which was five times greater than it is now. Explaining the present state of the asteroids and their origin is still a major task in Solar System science. □

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Cell biology

Traffic control and structural proteins in the eukaryotic nucleus

from Larry Gerace

ALTHOUGH RNA and proteins are exchanged continuously between the nucleus and cytoplasm in most eukaryotic cells, these two compartments each maintain a distinctive macromolecular composition. The nuclear envelope, the double membrane structure that forms the boundary of the nucleus, is thought to be a major regulator of these differences. Results discussed at a recent meeting* and in a number of publications augur a new period of progress for understanding the regulation of nucleocytoplasmic traffic, as well as the molecular organization of the nuclear envelope and lamina.

Protein import

Molecules are translocated across the nuclear envelope through proteinaceous channels called nuclear pore complexes (see the figure), which are probably the major regulatory sites for nucleocytoplasmic transport. The pore complex has an elaborate architecture that has been characterized by three-dimensional reconstruction from electron micrographs¹. Whereas it contains a mass that may exceed $25 - 50 \times 10^6$ daltons, almost nothing is known about its polypeptide composition. From microinjection studies, it is known that these proteins that are much more concentrated in the nucleus than the cytoplasm ('karyophilic' proteins) possess information in their mature polypeptide structure that is responsible for their accumulation in the nucleus^{2,3}. Since the pore complex seems to contain an aqueous channel with a functional diameter of about 10 nm (ref. 4 and R. Peters, Max

Planck Institute for Biophysics, Frankfurt), the rapid *in vivo* uptake of small (relative molecular mass, M_r , less than about 20,000 – 40,000) globular karyophilic proteins by the nucleus can, in theory, be explained by passive diffusion through the pore complex followed by intranuclear binding. The size of larger macromolecules, including many proteins and RNAs, would severely restrict their passive diffusion, and their translocation across the nuclear envelope may require a mediated-transport mechanism⁵.

Considerable effort is now being focused on identifying the signals that direct some proteins into the nucleus. A karyophilic protein that has proved to be a good model for studying mediated transport is nucleoplasmin, a 165 M_r pentameric molecule. Using limited proteolytic digestion, it has been determined⁶ that the nucleoplasmin monomer has a discrete 10 M_r 'tail' that contains the information mediating the selective nuclear uptake of this protein (R. Laskey, Cambridge University). In a striking experiment⁶, injection of nucleoplasmin-coated gold particles into the cytoplasm of frog oocytes has been shown to result in rapid nuclear uptake through the pore complex of particles with diameters as large as 20 nm (C. Feldherr, University of Florida, Gainesville). This suggests that the signal in nucleoplasmin causes the pore complex to become a 'gated' structure.

Recombinant DNA procedures have precisely defined the nuclear localization signal in the simian virus 40 T antigen^{7,8}, another large (94 M_r) karyophilic protein. A short sequence of the T antigen molecule (amino acids 126 – 132) is both suffi-

cient and necessary for the nuclear localization of this protein, and if this sequence is experimentally attached to large proteins that are normally cytoplasmic, it can retarget them to the nucleus (A. Smith, Integrated Genetics). Because a similar sequence is found in other viral nuclear proteins, it may be a prototype for one class of nuclear localization signal. However, as shown for the yeast MAT $\alpha 2$ protein, two distinct sequences on different regions of the same polypeptide can each be sufficient for nuclear uptake (ref. 9 and M. Hall, University of California, San Francisco).

The key issue to be addressed at this point is the nature of the cellular structures that recognize the protein signals for nuclear accumulation. Are they part of the nuclear pore complex or are they binding sites (chromatin or otherwise) within the nucleus? When the T antigen is expressed in cells of the alga *Acetabularia*, it also becomes concentrated in the nucleus (G. Neuhaus, Max Planck Institute for Cell Biology, Ladenburg), suggesting strong phylogenetic conservation of the nuclear uptake apparatus for this protein.

An interesting mechanism that is used by cells to regulate import of nuclear proteins has come to light from developmental studies with *Xenopus*. The proteins that are complexed with U2 RNA to form a small nuclear ribonucleoprotein particle (snRNP) are stockpiled in the cytoplasm of *Xenopus* oocytes in the absence of associated RNA, and only accumulate in the nuclei of embryos after transcription of the genes for U2 RNA starts at the mid-blastula stage of development¹⁰. Microinjection of U2 RNA into oocytes induces assembly of these U2-binding proteins into snRNP, which then migrate into the nucleus.

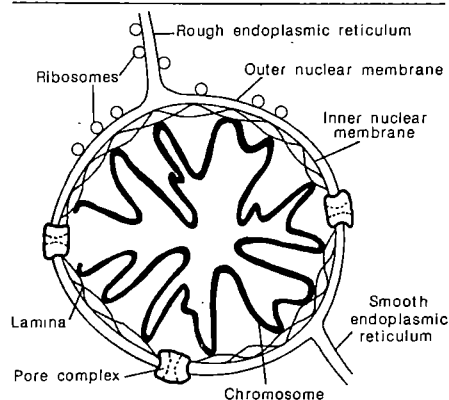


Diagram of the major structural components of the nuclear envelope, consisting of inner and outer nuclear membranes that are joined at the pore complexes, proteinaceous supramolecular structures that provide channels for molecular movement between the nucleus and cytoplasm. The outer nuclear membrane frequently has ribosomes attached and is structurally continuous with the rough and smooth endoplasmic reticulum. The nuclear lamina, a filamentous protein meshwork lining the nucleoplasmic surface of the nuclear envelope, probably provides an anchoring site at the nuclear periphery for interphase chromosomes.

*The Workshop on Nucleocytoplasmic Transport was held in Heidelberg, FRG, 26 – 28 September, 1985.

Mutant forms of U2 RNA that are not assembled into normal snRNP do not cause nuclear accumulation of the same proteins (ref. 11 and I. Mattaj, University of Basel). This indicates that karyophilic 'domains' are generated by RNP assembly, perhaps by conformational change. A variation on this mechanism may explain how certain proteins that are initially present in the nucleus of *Xenopus* oocytes become cytoplasmic during early cleavage stages of embryogenesis, and re-enter nuclei only at precisely defined periods later in development¹².

Encouraging progress was reported on the development of cell-free systems to study nuclear uptake of large proteins. Starting with isolated DNA or sperm chromatin and extracts of *Xenopus* eggs, several groups have assembled nuclei *in vitro* based on previous procedures^{13,14} which can accumulate nucleoplasmin (T. Burglin, University of Basel), nucleoplasmin-coated gold particles (R. Laskey) or endogenous karyophilic proteins of oocyte nuclei (C. Dreyer, Max Planck Institute for Developmental Biology Tübingen). Uptake of nucleoplasmin requires ATP both as *in vivo* as well as *in vitro* (T. Burglin), which is consistent with the operation of an active transport mechanism. Cell-free systems should offer a powerful approach for studying nucleocytoplasmic transport, particularly when immunological probes to the nuclear pore complex become available.

Lamina revealed

The nuclear lamina is a supramolecular assembly that lines the inner surface of the nuclear envelope and may both regulate nuclear envelope structure and anchor interphase chromosomes at the nuclear periphery¹⁵. In cells of several higher eukaryotes, the lamina consists mainly of a small number of related 'lamin' polypeptides. At least in *Xenopus*, these proteins are coded by a developmentally regulated gene family¹⁶, and the major lamins found in germ cells are distinct from the lamins of many somatic cells (G. Krohne, German Cancer Research Centre, Heidelberg).

Three discrete 60–70 *M_r* lamins (A, B and C) are present in typical mammalian somatic cells. The first amino-acid sequences of lamin A (G. Blobel, Rockefeller University) and, independently, of both lamins A and C¹⁷ have recently been deduced from human cDNA clones. Interestingly, a region of about 300 amino acids near the amino terminus of the lamins possesses striking sequence homology (greater than 80 per cent in certain regions) with the canonical 300-amino-acid, coiled-coil, alpha-helical rod domain that characterizes all intermediate filament proteins. A large domain with little alpha helix is predicted to occur at the carboxy terminus of the lamins. Electron microscopy in my laboratory (Cohn, J. & Gerace, L. in preparation)

shows that the three lamins from rat liver (which are apparently dimers) each contains a globular head attached to an approximately 50 nm rod-like tail. The latter bears close resemblance to the alpha-helical rod of intermediate filament subunits. Together with a recent demonstration that the lamina of *Xenopus* oocytes consists of filaments with a diameter of 8–10 nm arranged in a regular near-tetragonal lattice (Buhle, L. & Aebi, U. in preparation), these data indicate that the lamins constitute a hitherto unrecognized class of intermediate filament proteins.

Unlike many intermediate filament proteins of the cytoplasm, the lamins undergo total structural reorganization during mitosis in mammalian cells, when they are reversibly depolymerized to a 4–5S form by a process that may involve lamin hyperphosphorylation^{18,19}. An attractive possibility is that reversible disassembly of the lamina regulates the disassembly and reconstruction of the nuclear envelope that occurs during mitosis in higher eukaryotes. In support of this notion, I reported that removal (by immunoadsorption) of the disassembled lamins from a cell-free nuclear envelope assembly system results in strong inhibition of subsequent nuclear envelope formation. Mitotic-like disassembly of isolated nuclei can be induced by extracts from *Xenopus* eggs arrested in metaphase^{19,20}. (J. Newport, University of California, San Diego) suggesting that purification of lamina disassembly factors is feasible. Thus analysis of lamina disassembly may ultimately shed light on the biochemical regulation of mitotic prophase which, in one intriguing scenario²¹, may involve a phosphorylation cascade.

Only the lamina of higher eukaryotes has been characterized biochemically, but

it is also present in at least some lower eukaryotic organisms; indeed, it was first described structurally in *Amoeba proteus*. It is conceivable that the lamina emerged early during eukaryotic evolution, perhaps to carry out the functions of anchoring chromatin to the nuclear envelope and regulating nuclear envelope growth. The lamins may therefore represent an ancient class of intermediate filament proteins. Although the last in the current line of intermediate filament proteins to be recognized, they are certainly not the least interesting. □

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Palaeoclimatology

Cycles in the Precambrian

from A. Barrie Pittock

CLAIMS for the influence of sunspot cycles on the weather are highly controversial^{1–3}, so clear evidence for such an influence is an exciting prospect. Our best source of information is a series of periglacial varves in deposits from lakes in Australia. These varves are layers of silt laid down annually in a lake below the outlet of a glacier. They were first documented in 1981 by Williams⁴ from layering observed in a short section of exposed rock at Pichi Richi Pass in the Flinders Ranges. On page 523 of this issue G.E. Williams and C.P. Sonett present data from several drill cores giving a continuous stratigraphic sequence spanning some 19,000 'annual' layers⁵ which provides overwhelming statistical evidence that such cyclical activity really existed over a long period in the geological past.

Ever since sunspot cycles were first documented by Heinrich Schwabe in 1844, the possibility that they influence weather and climate has fascinated amateur and professional meteorologists^{6,7}. Claims for solar cycles in meteorological data have usually not been strong enough to overcome the variance in the data or to be obvious in visual inspection of time-series plots — the exceptions have in general been found to be spurious. Numerous small and marginally significant cycles have been proposed as the result of the statistical massaging of large quantities of meteorological data but most of these have again proved to be either spurious or at best controversial. Indeed, the noted Russian meteorologist A.S. Monin has characterized the field as one that is remarkable for "successful experi-

ments in autosuggestion"¹⁰.

The cyclical appearance of the varve sequence from Pichi Richi Pass is neither weak nor statistically marginal. Rather, it is visually obvious, and indeed dominates the total variance in the time series. The analysis by Williams and Sonett shows an impressive series of periodicities, including a basic cycle of approximately 12 'years', with alternating thick and thin cycles, and longer-term rhythmic fluctuations in the period every 13 cycles. Spectral analysis reveals a harmonic series with a fundamental period consisting of about 26.1 of the 12-'year' cycles and higher harmonics at 13.1, 8.8, 6.6 and 5.3 cycles.

Although the evidence for cycles in the varve sequence is overwhelming, there are problems concerning any interpretation of its physical significance. It is difficult to imagine any other explanation for the layered deposits than that of an annual meltwater cycle but the authors put 'years' in quotation marks, presumably to indicate that they have nothing more than circumstantial evidence for the annual origin of the layers. Similarly, the identification of the cyclical appearance with cycles of solar activity is largely circumstantial; we have no direct evidence for the cyclic behaviour of solar activity some 680 million years ago, so correlation of varve cycles with solar activity can only be hypothetical. Indeed, the authors imply that in future papers they will use information derived from the varve sequence to help with modelling solar behaviour — a procedure that would introduce a strong element of circularity into the argument.

Williams and Sonett interpret the varve sequence as indicating that increases in solar activity caused corresponding increases in climatic temperature, resulting

in greater annual meltwater discharge and the deposition of thicker varves. This is not consistent with a direct relationship between solar irradiance and surface temperature, since satellite measurements indicate that at times of increased solar activity, as indicated by sunspots, there is a slight decrease in solar irradiance¹¹. Of course, temperature at a particular locality on the Earth may be influenced by topographic or other local effects and so may not vary directly with fluctuations in solar irradiance.

Perhaps the biggest question posed by the varve sequence is why cycles should be so obvious in rocks about 680 million years old, when they are not strongly in evidence in the modern record. Williams and Sonett cite the existence of "a relatively weak solar signal" in recent varves from Skilak Lake in Alaska¹² but if this is the best evidence for the influence of solar cycles that can be found in recent times it leaves much to be explained. □

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100 years ago

THE Government of Tasmania are making arrangements upon a large scale for naturalising lobsters, crabs, turbot, brill, and other European fishes in the waters of that country. The various consignments will be shipped at Plymouth, and transported through the medium of the steamship companies trading between London and Hobart. An exhaustive report has been published by the Government of Tasmania, setting forth the objects in view, and giving suggestions for carrying them into effect. The report adds that while the achievement of the acclimatisation of European fishes would lay the foundation of new and very valuable fishing industries in Tasmania, it might also prove a highly remunerative commercial enterprise to the shipping firms under whose auspices the operations will be conducted. Applications have been made for the supplies of fish, which have been satisfactorily responded to. Special tanks are being prepared in order to provide for the necessities of the fish *en route*.

From *Nature* **33** 137, 10 December, 1885.

are associated with the activation of *c-myc* disrupt its regulation? One possibility is that they place *c-myc* under the control of a constitutively active gene enhancer so that it is no longer responsive to the normal cellular controls. This is consistent with the finding that the normal *c-myc* allele in tumour cells containing one activated *c-myc* gene is almost always silent^{6,9}.

An alternative possibility is based on highly suggestive evidence implicating the 5' exon of the cellular gene in its regulation. This exon, which is not translated, is missing from the *v-myc* oncogene that is found in some tumour viruses and is very often lost from the cellular gene as a result of the rearrangements in tumour cells. This has led to the proposal¹⁰ that *c-myc* is under negative regulation acting on the 5' end of the gene or its flanking sequences through the binding of a repressor protein. The experiments of Adams *et al.*, however, now show that the activation of *c-myc* is almost certainly independent of the presence or absence of 5' sequences; while the most recent evidence on the normal regulation of the gene suggests that the 5' exon may be important not in the control of *c-myc* transcription but in its post-transcriptional regulation.

The evidence that *c-myc* is normally transcriptionally regulated comes from measurements of *c-myc* mRNA. The induction of cell proliferation by mitogens dramatically increases *c-myc* mRNA levels², while the cessation of cell proliferation that accompanies differentiation leads to an equally dramatic reduction¹¹. These changes were initially assumed to be in the transcription rate of the gene. However, although a rapid induction of *c-myc* transcription is found in serum-stimulated fibroblasts¹², the 3–4 fold increase is not sufficient to account for the 20–40 fold increase in mRNA level. Furthermore, after a transient increase, the rate of transcription returns to the pre-induction level while the cytoplasmic RNA level remains high.

Oncogenes

Regulation and activation of *c-myc*

from Michael D. Cole

THE regulation of the *c-myc* oncogene and its role in the cancerous transformation of cells have been among the most widely studied and controversial subjects in the study of the molecular basis of cancer. This interest has been stimulated largely by the discovery that *c-myc* genes that have been activated by a variety of mechanisms (reviewed in ref. 1) are characteristic of a very wide range of tumours. Since the expression of *c-myc* is also characteristic of normal proliferating cells, however², it has become clear that in order to understand the nature of the abnormal activation of *c-myc* in tumour cells it is essential to understand how it is normally regulated. The paper by Adams *et al.* on page 533 of this issue³ provides important insight into the mechanism of abnormal *c-myc* activation and a number of recent studies have revealed an unexpected

complexity in the normal regulation of *c-myc* expression.

Two of the possible mechanisms of *c-myc* activation can now be dismissed. First, it is clear that the levels of *c-myc* messenger RNA are no higher in normal proliferating cells than in tumour cells, so that activation is not caused by overexpression⁴. Second, the expression of *c-myc* is constant throughout the cell cycle in normal cells⁵, so earlier suggestions that the gene is cell-cycle regulated in normal but not in tumour cells are not borne out. This has led to the alternative suggestion that activation involves the loss or disruption of control elements that enable the normal gene to be switched off when the cell enters the quiescent, non-proliferating state. The question then becomes, how do the chromosomal rearrangements and retroviral insertions that

More recently it has been established that the major cause of rapid changes in *c-myc* mRNA levels is a modification in post-transcriptional RNA stability. The levels of *c-myc* mRNA decrease rapidly both in lymphoblasts treated with interferon¹³ and in teratocarcinoma cells induced to differentiate with chemicals¹⁴. This occurs without any change in the rate of transcription, possibly because of a substantial decrease in the already short half-life of *c-myc* mRNA¹⁵. An increase in mRNA levels without a change in transcription rate is also seen in secondary lung fibroblasts¹⁶. In the light of recent evidence for a role for 5'-untranslated sequences in posttranscriptional regulation, it seems possible that the untranslated first exon of *c-myc* is the major target for destabilization or transport of the transcript. Post-transcriptional regulation at the level of RNA turnover therefore seems to account for much of the modulation of *c-myc* expression. These findings, however, still do not account for *c-myc* activation by the many proviral insertions and translocations that do not affect *c-myc* RNA structure.

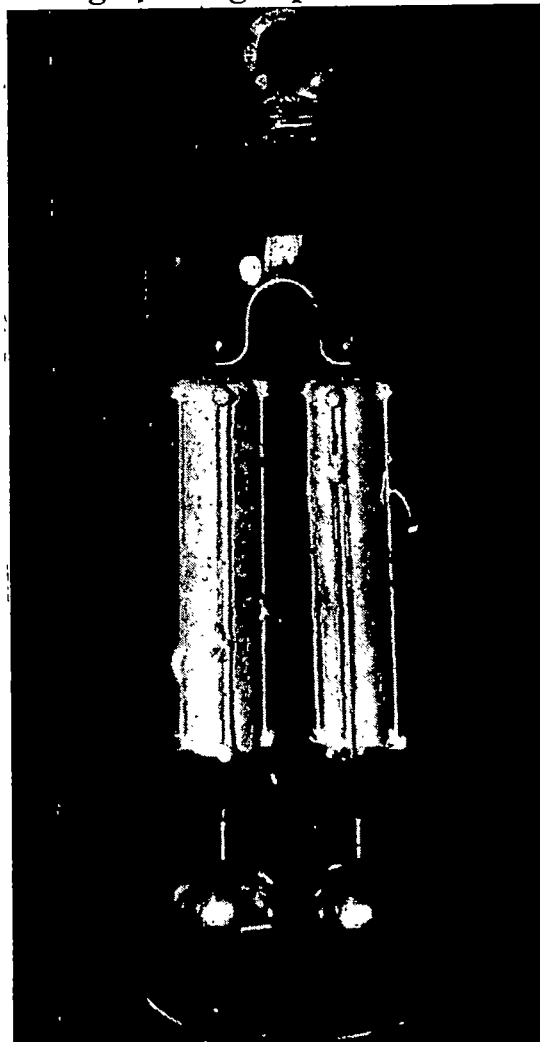
Adams *et al.*³ have addressed this problem using transgenic mice carrying different configurations of normal *c-myc* or *c-myc* genes linked to enhancer sequences from immunoglobulin genes. The results are quite striking and provide an elegant demonstration of the transforming potential of activated *c-myc* genes. Injection of *c-myc* genes joined to either the immunoglobulin heavy-chain or light-chain enhancers led to the induction of multifocal lymphomas in 90 per cent of the mice, while intact or truncated normal *c-myc* genes (without enhancers) produced no neoplastic disease. These findings are analogous to those of Stewart *et al.*¹⁷ in which a *c-myc* gene linked to a 'long terminal repeat' sequence of the mouse mammary tumour virus was shown to potentiate mammary carcinomas. In both studies, a tissue-specific control element apparently induces high levels of *c-myc* expression leading to a heritable susceptibility to tumours. Adams *et al.* clearly demonstrate that enhancement or transcriptional activation of *c-myc* is necessary to induce its oncogenic potential, while neither the alteration of its chromosomal position nor its truncation are sufficient for activation. However, this study does not provide an explanation of the mechanism of activation of *c-myc* transcription in the majority of Burkitt lymphomas and mouse plasmacytomas, where the immunoglobulin enhancer and other sequences that are known to control immunoglobulin gene expression are not present on the chromosome containing the *c-myc* coding exons and thus cannot influence the expression of the translocated gene. Presumably there is some unidentified transcription-activating sequence or change in chromatin configuration in these other cases.

By analysing not only *c-myc* expression

but also immunoglobulin rearrangements, Adams *et al.* obtained important additional information. First, the majority of the tumours (which were of both pre-B and B-cell stages) had a distinct set of rearranged immunoglobulin genes, which indicates that the tumours were clonal. Because the presence of the immunoglobulin-enhancer-linked *c-myc* gene in the germ line should lead to high levels of *c-myc* expression in all lymphocytes, this result suggests that a second event may be required to generate a fully transformed cell.

Furthermore, Adams *et al.* find that, whereas the enhancer-linked *c-myc* gene is expressed in the tumours, the endogenous *c-myc* gene is not. This makes the induced tumours analogous to natural tumours with transcriptionally activated *c-myc* genes, such as plasmacytomas^{18,19} and Burkitt lymphomas^{8,9}. In the light of the transcriptional and post-transcriptional control of *c-myc* expression discussed above, it will be very interesting to learn at what level the endogenous gene is down regulated. Adams *et al.* argue that the lack of endogenous *c-myc* expression is consistent with an autoregulation control model, in which expression of the activated allele suppresses the expression of the germ-line gene. However, as the authors note, they cannot exclude the possibility that the transformed B cells in their study arose from a cell in which the endogenous *c-myc* gene is not normally expressed. Furthermore, autoregulation is not consistent with observations of expression of the endogenous *c-myc* gene in some of the mammary carcinomas induced by *c-myc* linked to the 'long terminal repeat' sequences of mouse mammary tumour virus¹⁸, nor with the effects of introducing a constitutively expressed *c-myc* gene into fibroblast lines that are already expressing *c-myc*; in this case, again, the introduced gene does not affect the expression or regulation of the

Long-running experiments (III)



A dry pile has driven this electric bell since sometime before 1840 when it was re-set up in Oxford University's Clarendon laboratory. The clapper draws about 1 nA as it oscillates between the terminals at a speed which declines with humidity. Occasionally humidity stops the movement, which starts again spontaneously. It is not known what the piles are made of. More details in *European Journal of Physics* 5, 193; 1984, which invites "accounts of other experiments that call for patience on the part of the recorder".

endogenous gene¹⁸.

Is it possible that the 'second event' that seems to be required for transformation normally turns off the *c-myc* gene in a manner analogous to that seen in terminal differentiation, and that DNA rearrangements or proviral enhancers prevent this down regulation? Despite the progress that has been made in understanding the regulation of this complex and interesting oncogene, clearly many challenging questions are still unanswered. □

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Mathematics

Classical continued fractals

from Ian Stewart

CONTINUED fractions are classical: they provide 'best possible' rational approximations to real numbers, expressed in the form $\alpha = 1/(a + 1/(b + 1/(c + 1/ \dots)))$, usually written as $[a, b, c, \dots]$ for simplicity. Their main use is in number theory. Fractals are of much more modern vintage. They are geometrical objects with structure on all scales. The name was coined by their inventor, Benoit Mandelbrot, to reflect their 'infinitely crinkled' shape. They are applied to irregular behaviour and form in nature.

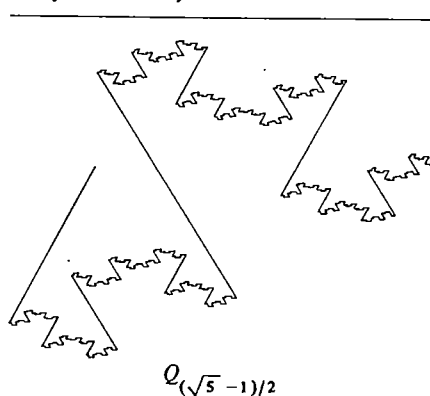
Can two such disparate ideas usefully be combined? Indeed they can. Jenny Harrison of the University of California at Berkeley has announced progress on a baffling problem known as the Seifert conjecture based on what she calls continued fractals. The proof is sketched in *Bulletin of the American Mathematical Society* 13, 147; 1985; full details will appear elsewhere.

The celebrated 'hairy ball' theorem states that it is impossible to comb a hairy ball smooth. More precisely, any smooth vector field on a two-dimensional sphere must have a fixed point. In consequence, any smooth dynamical system whose state space is topologically a two-dimensional sphere must have at least one steady state. On the other hand, a hairy torus can easily be combed smooth, so dynamics on a torus can have no steady states at all. Such results have far-reaching consequences: for example they justify the choice of tori, rather than spheres, for magnetic bottles in experimental fusion reactors.

But what if the state space is a three-dimensional sphere? First we must explain how such an object can be visualized. The circle, a one-dimensional sphere, can be obtained from a line by adding a 'point at infinity' which is considered as lying at both ends, so that the line plus this point curls up into a circle. Similarly the 2-sphere can be thought of as a plane, plus a single point at infinity, lying at both ends of every line in the plane. The 'horizon circle' of the plane is squashed down to this single point; and much as a bag can be closed by tightening a loop around the top, the plane curls up to give a sphere. For a 3-sphere, just add a single point at infinity to three-dimensional space. To

visualize a smooth combing of the 3-sphere, we imagine a family of smooth curves through 3-space, taking care that it behaves suitably 'near infinity'.

It is not hard to find ways to comb a hairy 3-sphere smoothly, with no fixed points. Other special features tend to appear, however, notably places where the hairs form a closed loop. Is there a way to comb a 3-sphere that has no fixed points and no loops? In 1950, Herbert Seifert conjectured that there is not or, more precisely, that every smooth vector field on



Example of a continued fractal (redrawn from Harrison, J. *Bulletin of the American Mathematical Society* 13, 147; 1985).

the 3-sphere has either a fixed point or a closed integral curve. At first, very little progress was made on this fundamental problem, because nobody knew where to start.

To describe the known results we must address the question of how smooth is 'smooth'? The degree of smoothness of a curve is measured by how many times it can be differentiated. To qualify as 'smooth' it must certainly have a tangent everywhere (be once differentiable). If the position of that tangent also varies smoothly, the curve is twice differentiable, and so on. The smoothest curves are infinitely differentiable. A fancier definition lets the degree r of differentiability be fractional, indeed real, as well as integral.

In 1971, Paul Schweizer (*Annals Mathematica* 100, 386; 1971) showed that the Seifert conjecture is false in the once-differentiable case. So for a degree of differentiability r somewhere between 1 and ∞ , there is a switch from false to true (unless, as may be the case, it is always false). The question is, where? In her thesis under Colin Rourke at Warwick University, Harrison showed that the switch is at a value $r > 2$. Her new work, which uses similar methods, improves this to $r \approx 3$, pushing current techniques somewhere near their limit.

The starting point of her method is a

deceptively simple example of discrete dynamics. Take a circle, select a real number α between 0 and 1, and define a transformation T of the circle by rotating it through the angle $2\pi\alpha$. Then, under iteration of this transformation, the behaviour of points is highly sensitive to the degree of irrationality of α . Specifically, suppose α has continued fraction $[a, b, c, \dots]$, then the smaller that the entries a, b, c, \dots are, the more irrational is α .

When the rotation α is rational, every point on the circle is periodic under iteration of T . That is, if T is performed sufficiently often, every point ends up exactly where it started. If α becomes irrational, this periodicity starts to break up. For highly irrational α , such as the golden number $[1, 1, 1, 1, \dots]$, the iterates under T are evenly spread over the circle. For 'almost rational' numbers, like the Liouville number $[1, 2, 3, 3^3, \dots]$, the points tend to clump together during long sequences of iteration. Thus there is a strong link between the dynamics of T and the continued fraction of α .

There are well known techniques for constructing flows on the 3-sphere from transformations such as T , and the central question is to determine how the degree of differentiability of the resulting flow depends on the degree of irrationality of α . The falsity of the Seifert conjecture unless $r > 2$ comes from taking $\alpha = [4, 4, 4, \dots] = \sqrt{5} - 2$. Can other choices of α lead to smoother flows?

It is here that continued fractals enter. Harrison describes a geometric way to visualize the continued fraction of α , by 'unfolding' the sequence of iterates of a point under the rotation T . This leads to a curve whose geometric structure determines arithmetical properties of the continued fraction. The curve is a fractal, and its geometric fine structure corresponds to delicate arithmetical properties, making it possible to visualize the way in which α is approximated by rationals, and to apply geometric reasoning. The improved value $r \approx 3$ arises by taking $\alpha = [N, N, N, \dots]$ for large and even values of N , and by exploiting the interplay between dynamics, geometry and arithmetic. It is a vivid display of the unity of mathematics.

Meanwhile, the general Seifert conjecture remains as enigmatic as ever. Conceivably it is false even in the infinitely differentiable case. There seems little chance that current techniques can lead to a general counter-example. Harrison's methods break down for r larger than 3, leading her to suggest that it is, at $r = 3$, where the conjecture turns into a theorem. However, no proofs exist for any r, ∞ included; other considerations suggest that a proof (if indeed the conjecture is ever true) must overcome formidable technical obstacles. We may have a long wait for the complete answer. □

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Errata

Throughout A.M.C. Sengor's article "East Asian tectonic collage" (7 November, p.16) the term "Indosinian" should replace "Indonesian", which was inadvertently substituted during editing.

In Michael Brown's article "Tectonics of metamorphism" (28 November, p.314), the wrong photomicrograph was used to illustrate Fig.2.

Another reason for saving Sakharov

SIR—I believe it is crucial in our attempts to rescue Andrei Sakharov to re-emphasize Sakharov's scientific legacy to the scientific community at large and to Soviet officials in particular. Studying recently an early paper on gravitation theory by Sakharov¹, I realized that this work not only provides a new way of looking at gravity, but also gives us a new weapon in the battle for Sakharov's life. The paper, which describes the possible connection between gravitation and vacuum fluctuations, has been referred to in a number of major textbooks²⁻⁴. My own investigations have given me an even higher opinion of this seminal paper.

I have been studying the influence of a suggested inhomogeneity in zero-point vacuum fluctuations on free particle motion and have come to the conclusion^{5,6} that in the general case the particle world-lines become geodesics of a Riemannian geometry. So I was able to appreciate Sakharov's main suggestion of a direct connection between the vacuum fluctuations and gravity (that is, Riemannian geometry of spacetime). In the appropriate sense, Sakharov obtains the Hilbert Lagrangian of general relativity. As a result the Einstein equations acquire the meaning of connection between an inhomogeneity in the vacuum fluctuations and the distribution of matter. Some formal difficulties in the development of Sakharov's concept are removed by the recently derived covariant description of gravitational energy.

I believe that Sakharov's concept may resolve the 60-year confrontation between quantum field theory approaches to gravity and Einstein's general relativity, after hundreds of futile attempts to bridge this gap. Once some formal difficulties are removed, I anticipate the development of the physics of vacuum fluctuations as a fundamental branch of science with many applications. Hence I believe that historically Sakharov's name will be among the most illustrious names in science. Like Evariste Galois, another genius of the highest originality, Sakharov chose the way dictated by his moral self-obligation and this deprived him of the opportunity to advance many of his ideas. Like Galois, he gave only a short synopsis of his concept. I believe that the concept will steadily increase in importance to science, as did Galois' contribution.

Hence I perceive a new starting point in our efforts to save Sakharov's life. His humanitarian activities have been completely rejected by Soviet authorities as part of the dangerous "bourgeois ideology" with which they claim no conciliation is possible. They do not think that Sakharov's role in the creation of Soviet nuclear weapons is worthy of reward or respect in the West. He is not considered as a head of

some scientific school inside the Soviet Union or abroad. So, to Soviet authorities, Sakharov is nothing but a Western tool of the cold war. They assume that he will be forgotten in the West as soon as he is out of sight. Hence, from their point of view, Sakharov may safely be ignored. Perhaps the new Soviet leaders will rectify this situation when they realize that his scientific legacy will not be forgotten by history and that Sakharov's persecution will be recalled in every physics textbook.

It is important to make it clear to the Soviet authorities (and perhaps to some of us also) that Sakharov is likely to be seen as the founder of a new fundamental branch of physics which may find wide application. I believe that the best way to advance this understanding is the rapid development of Sakharov's concept and its broad explanation. My belief is that the investment of money and intellectual resources in Sakharov's concept are completely justified by the expected scientific results. But there is an additional justification. The rescue of Sakharov is our ticket of admission to the human race.

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Ocean thermal response in man-made climatic change

SIR—John Woods has raised¹ important issues concerning the dynamic response of ocean circulation to anthropogenically imposed changes in atmospheric CO₂ level. The full implications of the physical characteristics he presented have potentially serious environmental policy consequences beyond those discussed in his paper. The delay in ocean-atmospheric temperature equilibration due to the ocean's thermal mass following the introduction of man-made infrared-absorbing trace gases into the atmosphere implies a slower rise in global climatic warming than given by calculations that omit oceanic effects. The time it would take to reach the conventional measure of a significantly hazardous environmental condition (a global temperature rise of about 3°C, which corresponds to doubling of atmospheric CO₂ concentrations to about 600 p.p.m.v.) can thereby be lengthened by from 20 to 100 years².

Woods suggests that such a delay in temperature rise will ease the potential for traumatic climate change by providing extra time for initiation of measures to reduce climatic warming, such as the

accelerated development of nuclear in place of fossil energy. But this likely will not be the course of events. Rather, it is more probable that the needed remedial actions will be delayed until the observed climate change can be unequivocally identified with atmospheric CO₂ growth, by which time its concentration could have risen above a value whose ultimate equilibrium climate effect would be calamitous. From this perspective, thermal inertia of the oceans introduces a type of irreversibility which is obviously of major importance for policy, since global temperatures will continue rising even after a decision to drastically reduce fossil fuel use has been implemented.

Scientists' response to this dilemma is naturally to suggest that predictions, rather than observations of temperature change, be used as the basis for action. But this requires hard political decisions which are made more difficult by the acknowledged lack of understanding and quantifiability of the physical phenomena concerning climatic change and its impacts. How to properly bring the relevant but uncertain scientific information into the decision-making process, is important, and attention is starting to be paid to this type of need^{3,4}.

Before we conclude that existence of long thermal ocean inertia lags inevitably results in the major irreversibility dilemma presented above, we should point out a revision in the usual viewpoint that is needed in order to properly address the physics of the problem. The ocean transient response is normally described in terms of an e-folding time for approach to thermal equilibrium — setting atmospheric CO₂ level to be constant. However, from a policy viewpoint we need to follow the approach to a steady state following elimination of CO₂ emissions. All current carbon cycle models predict only a modest fall off rate of CO₂ levels following such a step⁵, but these probably do not allow for the involvement of deeper ocean layers that results in the extended e-folding times we have quoted. In fact, it seems intuitively clear that stronger coupling to the deep ocean implies a larger short-term oceanic sink for CO₂ deposition, and hence a faster fall of atmospheric CO₂ concentration following reduction of CO₂ emissions. If this qualitative surmise is true (and an analysis of it has yet to be made), the irreversibility feature of the policy issue we have raised may not be as severe as it seems at first glance.

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Misuse of statistics in social sciences

SIR—Two letters, one from Van Valen¹, the other from O'Quigley and Baudoin², have warned against the indiscriminate use of null hypothesis testing and the confusion of level of significance with scientific certainty. The situation is worse even than they make it seem, at least in the social sciences.

As Van Valen points out, R.A. Fisher developed the analysis of variance not for hypothesis testing but to aid scientific questioning. Instead it is used in the social sciences to come to scientifically irrelevant decisions about null hypotheses. My alpha (the level at which I reject the null hypothesis), cautious person that I am, is always one in a million. Yours is one in ten. We both live by the rules. You get a lot of significant results (or almost so, or highly so); I get none. Who is right scientifically?

Guttman³ argues that hypothesis testing as used in the social sciences is, strictly speaking, anti-scientific. Science is about the accumulation of information and testing by replication. Hypothesis testing is used to dodge replication and come to immediate decisions. Yet one test of significance leaves indeterminate the probability of the outcome of the next test on a new sample. In other words tests of significance are not cumulative and do not remove the need to replicate. The result is a landscape dotted with isolated studies forming no pattern and making no collective sense. Guttman also points out what is obvious on reflection, that simultaneous *F* tests on rows, columns, interactions and so on, routine in the social sciences and built into packages such as SPSS, encounter the same problem that simultaneous tests do; they are not independent. The same error flutter that makes one contrast improbable can affect another. The logic of hypothesis testing for any other than the one-way analysis of variance is flawed.

The Neyman–Pearson statistical tools are framed in terms of a loss function: the money or resources that a decision will cost. They are designed to minimize losses in gambling, farming, industry and war. It was gamblers who first demanded probability theory and agriculturists who adopted inferential statistics. Their common problem was the need to make decisions under risk. Social scientists, faced with difficult experimental problems, took up hypothesis testing *faute de mieux* to make progress rapidly and to lend their endeavours the appearance if not the reality of scientific respectability. It is in fact irrelevant to science which is concerned with making sense, not conserving resources.

The bankruptcy of programmes of research based on the crutch of the *F* test is revealed by the choice of the null hypothesis. It is almost always that a correlation or a mean difference is zero. If knowledge were accumulating the incumbent hypothesis would hardly ever be that of no correlation or no effect. Yet this is what is tested routinely, as if all that had gone before counted for nought. It is correct to be dubious about the claims of little old ladies to tell by taste how cups of tea were poured, milk first or second. We can calculate probabilities and find them out, just as we can detect fudging, by Mendel and by Burt. The hypothesis of no effect is the correct one for improbable claims, of psychokinesis or water divining, for example. But it is not correct when we have a body of knowledge to draw on and a theory designed to predict not just that something will happen but what and how much.

The fault does not lie with statisticians. Their job is to tell us how to do statistics, not science. It is scientists who have to work out how to do science. By misunderstanding the scientific use of statistical tools social scientists have created a situation every bit as bad as Guttman says it is. Puttering along, making the same old mistakes, will in the end bring the whole enterprise into disrepute.

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No to new photosynthetically active radiation units

SIR—In response to R.A. Lewin (*Nature* 316, 582; 1985), who proposes a new unit to measure photosynthetically active radiation, certain misconceptions should be dispelled. The photosynthetically active photon flux density (PPFD) is the quantity of photons incident on a unit area per unit time. It is not, as claimed, light intensity which is the luminous flux emitted by a point source into a unit solid angle (SI unit, cd), nor is it uniquely PAR (photosynthetically active radiation) which is commonly used for the radiant power (400–700nm) incident on a unit area per unit time (SI unit, Wm^{-2}).

Communication between ecophysiologists has been greatly aided by the adoption of SI units. A particular advance has been the use of PPFD (units: $\mu\text{mol m}^{-2} \text{s}^{-1}$). This unit is consistent with those of O_2 , CO_2 and H_2O fluxes, greatly simplifying the calculation of derived parameters such as quantum efficiency of efficiency of water use.

There are limitations to the use of PPFD. Density is confusing for a flux through a unit area rather than volume. The term is biased to land plants being

limited to the photosynthetically active wavelengths (400–700nm). However, the 'alb', the new unit proposed by Lewin, solves none of these problems and only adds to the confusion.

Lewin also complains that PPFD requires the use of the Greek letter, μ , which is awkward to type. Awkward as it may be, modern scientific word-processing packages can easily solve this problem. In any case, SI derived units are composed of base units, thus the 'alb' would be $\text{mol m}^{-2} \text{s}^{-1}$, and avoidance of negative exponents, abhorred by Lewin, would require the use of the ' μalb '. Does the author object similarly to μg and μm ? Plants, especially algae, differ in their absorption spectra, but unless sensors can be designed to fit each individual plant, there is no practical solution to this problem, and the alb provides no advance. If Einstein's first name be abbreviated to alb, let it be for an advance in science and not for a unit which solves no problems and adds to the confusion of light measurements.

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SIR—To contradict Lewin's recent letter suggesting the alb as a radiation unit, instead of 'einstein', I consider that there is no confusion in the physics community about the use of the einstein. The ubiquitous ratio (velocity)/(velocity of light) = v/c is commonly known as β , and is otherwise not named. The authority for this statement is (1) your nearest friendly physics text, (2) excellent physicists, (3) one not-so-excellent physicist (L.X.F.).

Einstein's contributions to physics are incomparably wide and basic. So many units would be justifiably named after him than none is.

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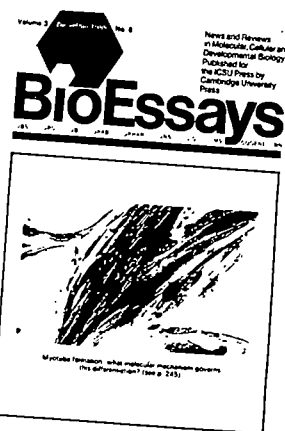
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Reader Service No.5

Healing in perversion

Michael Shepherd

The Breaking of Bodies and Minds: Torture, Psychiatric Abuse, and the Health Professions. Edited by Eric Stover and Elena O. Nightingale.
W.H. Freeman: 1985. Pp.319. Hbk \$21.95, £22; pbk \$11.95, £11.95.

TORTURE: "the art or process of inflicting severe pain, especially as a punishment, so as to extort confession, or in revenge". This is an impersonal dictionary definition of an unpleasantly personal topic, one which Voltaire saw as important enough to include in his *Philosophical Dictionary* of 1769 to underline its significance for the society of the day:

The Romans inflicted torture only on the slaves, but the slaves were not reckoned to be human. Nor does it appear that a judge . . . regards as a fellow man the haggard, pale, broken individual who is brought before him dull-eyed, with a long dirty beard, covered with the vermin that have been preying on him in the dungeon. He gives himself the pleasure of putting him to major and minor torture, in the presence of a surgeon who feels his pulse until he is in danger of death, after which they set to again. . . .

Until recently Voltaire's condemnatory views have been expressed by a relatively small minority, even though the twentieth century can claim some of the most savage examples of human barbarism in recorded history. On Human Rights Day in 1972, Amnesty International began its campaign against the systematic use of torture by governments, and in the following year published its disturbingly well-documented *Report on Torture* (Duckworth, 1973).

The *Report* included a full account of the medical and psychological aspects of torture and demonstrated by means of a survey that "torture has virtually become a world-wide phenomenon and that the torturing of citizens regardless of sex, age or the state of health in an effort to retain political power is a practice tolerated by others in an increasingly large number of countries". The topic has since attracted mounting attention and concern, though it was not until December 1984 that the Convention against Torture and Other Cruel, Inhuman or Degrading Treatment or Punishment was adopted by the United Nations General Assembly.

The psychological and psychiatric evidence, concluded the *Amnesty Report*, points to the existence of the potential to torture in *Homo sapiens*. Voltaire, who recognized torture as one aspect of the destructive element in man, would not have been surprised by these findings, while clinical observations have indicated that John Cowper Powys's admission that he could not remember a time "when sadistic thoughts and images did not disturb and intoxicate me" is unusual more by virtue of its frankness than by its con-

tent. Further, torture carries its own appeal. Writing of perhaps the most distressing scene in world literature, the blinding of Gloucester in Shakespeare's *King Lear*, Wilson Knight remarked shrewdly that, "The sight of physical torment to the uneducated brings laughter". And with the laughter goes a form of pleasure, widely exploited by pornographers and wholly unrelated to educational status.

The Breaking of Minds and Bodies, published under the auspices of the American Association for the Advancement of Science, should help carry the campaign against torture a stage further. Its subtitle, however, *Torture, Psychiatric Abuse, and the Health Professions*, is more informative than its title. The book is in two parts, one concentrating on general issues and the other on psychiatric practices in the Soviet Union, each containing five articles by various authors with several case-histories, along with editorial commentaries and three appendices providing codes of ethics, an inventory of relevant organizations and a selected bibliography. Much of this information is familiar and, indeed, three of the articles have appeared elsewhere. The principal purpose of the book, however, is not so much to record the activities of repressive governments as to explore how they have "enlisted the aid of medical practitioners in suppressing dissent and what steps need to be taken to prevent such professional complicity, and ultimately, to end the abuses themselves".

The message is all the more timely inasmuch as the involvement of the medical profession has not always been acknowledged. The medical profession, nominally devoted to the preservation of health and the saving of life, places great store on the ethical conduct of its members, as epitomized in the International Code of Medical Ethics, adopted by the World Assembly of the World Medical Association in 1949, which states categorically that "Under no circumstances is a doctor permitted to do anything that would weaken the physical or mental resistance of a human being except from strictly therapeutic or prophylactic indications, imposed in the name of his patient". In fact, as the evidence makes clear, medical practitioners continue to disobey this injunction, actively or passively, in many countries. Voltaire's pulse-taking physician, far from giving succour to the victims of torture, was participating in the process.

Why does this occur? The book focuses on psychiatrists in the Soviet Union, whose well-documented activities occupy a major part of the text. By employing their mental hospital system to coerce dissident but mentally healthy citizens and perverting clinical concepts for the purpose, the Russians have provided a new variation on an old theme. But anyone who has had contact with medical representatives of this system cannot fail to recognize, along with the cynical, unscrupulous apparatchik, the sincere, if misguided, believer in an ethical system codified in the Physician's Oath of the Soviet Union which, after mentioning the health of man and the care of disease, goes on to state: "I will in all of my actions be guided by the principles of communist morality, ever to bear in mind the high calling of the Soviet physician, and of my responsibility to the people and the Soviet state".

This chilling sentence draws attention sharply to the need for account to be taken of more than one set of ethical guidelines when assessing professional behaviour. As Heijder has pointed out (see *Professional Codes of Ethics Against Torture*, published by Amnesty International in 1976), such conduct is affected by at least four factors: political systems, public opinion, organizational units and individual codes of morality. The noxious doctor, like the immoral priest or the crooked lawyer or the dishonest scientist, is the product of all these forces, whose balance determines the answer to the question posed by a distinguished German-Jewish refugee who had left Germany early in 1933 after finding most of his colleagues at the university clinic dressed in Nazi uniform the day after Hitler's accession to power — in the event of such circumstances in this country, he would ask, how many of your colleagues would be wearing civilian clothes?

Clearly it is unnecessary to postulate a Joseph Mengele in the vast majority of those uniformed physicians. The central issue is how far association with or acceptance of any system which employs or condones torture in any form is compatible with a medical ethos which must be preserved if the profession is to maintain its public status and its self-respect. Stanley Milgram's disturbing experiments on obedience and disobedience in response to authority reinforce the fragility of personal conscience in conflict with social pressures and reinforce the verdict of the *Amnesty Report*, namely that "The prevention of torture . . . lies not in medical research but in political and legal remedies". The value of this book resides in its contribution to the heightening of public awareness and the facilitation of official action. □

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Between a good read and a good reed

John C. Marshall

Children's Reading Problems. By Peter Bryant and Lynette Bradley. Basil Blackwell: 1985. Pp.116. Hbk £16.50, \$24.95; pbk £5.95, \$9.95.

Reading Ability. By Charles A. Perfetti. Oxford University Press: 1985. Pp.282. £25, \$35.

IN 1945 Herbert Hoover declared that the liquidation of illiteracy was one of the five or six major tasks for the postwar world. It still is. Even in the industrialized world a small but distressing minority of children experience great difficulty in learning to read and never achieve the fluent command of visual language that is necessary to participate fully in a technological society; what can be done for the 'slow learner' or the 'dyslexic' child? Many students in tertiary education who have mastered the nuts and bolts of reading complain of slow reading speeds and poor comprehension; can these young adults learn to cope better with the demands that professional life places upon true literacy?

These two books reach a similar conclusion on one key issue: the invention of the alphabet was a good idea, and a firm grasp of the alphabetic principle that letters (or letter groups) map onto the constituent sounds of words is of crucial importance in learning to read. Beyond this, the books differ greatly in both style and content.

The primary virtue of Bryant and Bradley's monograph lies not so much in their conclusion *per se* (advocates of so-called 'phonic' instruction have been with us for a century or more), but rather in their evidence for that conclusion. First reported in this journal (*Nature*, 310, 419–421, 1983), Bradley and Bryant have provided the most compelling demonstration that awareness of the relationship between letters and sounds is *causally* implicated in the efficiency with which children learn to read.

Children's Reading Problems contains a vigorous, albeit good-natured, attack upon the methodological and interpretative inadequacies of much previous research; it defends, more cogently than any other work I know, the central importance of phonic skills. The book is written in lucid English, quite free of psychological jargon. Parents and teachers should find Bryant and Bradley's simple description of their training methods of considerable practical value. More generally, Bryant and Bradley provide an acute analysis of how to conduct serious educational research on which practical decision-making could be rationally based.

One aspect of their work is, however, controversial. They fail to acknowledge the full import of the fact that English (or

French) cannot be read or written solely on the basis of letter-sound correspondence rules. The child who writes, YOT, or PLAZE (for "yacht" and "plays") has mastered the skills that Bryant and Bradley stress; likewise, the child who reads SHOE as "show" or SWEAT as "sweet". But to attain mature literacy the child must eventually come to read and write orthographically and forgo reliance on phonic rules; a good read is not the same as a good reed. One alarming consequence of Bryant and Bradley's over-reliance on phonological awareness is their belief that reading backwardness must have a solitary cause — failure to acquire normally the regular mappings between letters and sounds.

This flies in the face of the extensive evidence, from single-case studies, that backward readers (or 'developmental dyslexics') constitute a very heterogeneous population with striking qualitative variation from child to child in the nature of their impaired and intact sub-skills. Bryant and Bradley are well aware of this literature (they quote a few pertinent examples), yet they dismiss its significance on the peculiar grounds that analogous qualitative variation can be found in younger children who read at age level. This seems such an obvious *non-sequitur* — why should the balance of skills required to read normally at one age be identical with that required for normal reading at a later age? — that one is deeply puzzled to see it put forward by *developmental* psychologists.

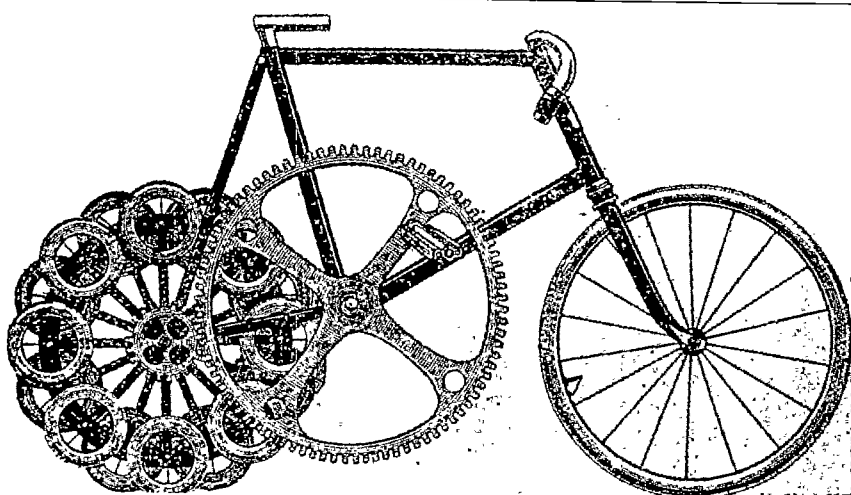
By contrast with the straight but narrow path chosen by Bryant and Bradley, Perfetti reviews a substantial chunk of the primary literature on all aspects of reading ability in both children and adults; the discussion ranges from word recognition to text comprehension, from the analysis of eye movements in skilled reading to the role of verbal short term memory, and from brain laterality to computerized reading instruction. Many of the studies

reported are as relevant to speech processing and oral comprehension as they are to reading and writing. These review chapters are solid and workmanlike, although somewhat heavy going; Perfetti's turgid style lacks the sparkle and excitement of Bryant and Bradley's clear arguments.

Perfetti's own "verbal efficiency theory" succeeds in showing that comprehension of a written text is a complicated business but provides little real insight into the mysterious processes of "propositional assembly" and "propositional integration". Much of this research has a curiously circular flavour. Groups of high- and low-ability readers are selected and then shown to differ on a variety of reading-related skills. The unproductiveness of such a strategy derives in part from the use of heterogeneous groups; it is thus unclear whether a particular impairment is characteristic of all low-ability readers or solely that of a subgroup. Many of the studies also fail to distinguish between cause and effect; an impairment of "verbal intelligence" could be a cause of poor comprehension, but equally the failure to learn from reading may be reflected in poor performance on other language tasks. In contrast, Bryant and Bradley's discussion of how to disentangle cause and effect is particularly incisive.

These problems assume major significance in Perfetti's chapter on dyslexia. He tentatively concludes that "dyslexia may be the low end of an ability continuum rather than a qualitatively different problem." But once again, the failure to distinguish individuals from groups renders the conclusion suspect. Indeed, the very concept of a single ability continuum seems misguided when we are dealing with cognitive functions, such as reading and writing, whose effective deployment depends upon a large range of subcomponents. □

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Some mistake? Thomas Bennett's "Eureka" driving wheel for bicycles of 1897, which, he boasted, made "practically a frictionless machine". The illustration is reproduced from *A Victorian World of Science*, by Alan Sutton, published by Adam Hilger price £12.50, \$20.

Into the generation of application

Karol Sikora

Hybridoma Technology in the Biosciences and Medicine. Edited by Timothy A. Springer. *Plenum: 1985. Pp.602. \$75, £71.*
Human Hybridomas and Monoclonal Antibodies. Edited by E.G. Engelman, S.K. Foug, J. Larrick and A. Raubitschek. *Plenum: 1985. Pp.526. \$69.50, £66.*

SINCE the discovery, ten years ago of monoclonal antibodies, several books about these biologically useful tools have been produced. Until now, however, they have tended to concentrate heavily on methodology. But we are beginning to see the emergence of second generation volumes which provide the reader with much more sophisticated detail on the application of the technology. These two books are in this category.

The first, edited by Timothy Springer from Harvard University, starts with a rather dry title. However, in it the editor has managed to get together a group of authors who take us through some of the more elegant approaches to the construction and characterization of monoclonal antibodies. Recent developments in hybridoma technology involved in the production of monoclonal antibodies — peptide immunization, the expression of cloned immunoglobulin genes, and exon shuffling and class switching — are all considered and fully referenced. I know of no other source to date where this information can be found in one place. The merger of gene cloning and the monoclonal antibody fields promises to bring mutual benefit. Designer monoclonal antibodies — that is those produced with the required secondary functions by recombinant gene technology, may now become a reality, whilst the speed of cloning defined genes can be aided by having an appropriate antibody to the resulting protein.

The second section consists of a series of reviews on the biomedical application of monoclonal antibodies. Some of these are rather unoriginal and now dated, especially those concerning clinical applications. The final section considers T-lymphocyte clones and their products. Unfortunately this field has moved too fast for the publisher and although there are some useful background reviews little recent information is to be found. Altogether this is an interesting volume, containing much useful reference material about new ways to produce and improve monoclonal antibodies, but it could easily have been shortened without detriment by removing some of the reviews on specific applications.

The production of human monoclonal antibodies has captured the imagination of many over the past five years. Their

advantages in allowing a direct examination of the human immune system and the potential lack of immune response to them on injection suggest that they will be more useful clinically than their rodent counterparts. But there are tremendous technical difficulties in their production and these problems are addressed by the contributors to the first volume specifically to be concerned with human monoclonal antibodies.

The technical problems and how they can be overcome are considered in the first part of the book, which provides recipes for mouse-human and human-human hybridization as well as the use of Epstein-Barr virus to achieve immortalization. The production of human antibodies to cancer cells, infectious agents and self antigens in autoimmune disorders such as diabetes and systemic lupus erythematosus are reviewed. An interesting mixture of chapters then considers specific aspects such as the study of human-human hybridomas in immunodeficiency, the use of electrofusion techniques and the generation of human

T-lymphocyte hybridomas. There follows an appendix listing methods for tissue culture and assaying supernatants and recipes for media. Lists such as these have little to do with human monoclonal antibody production specifically and are readily available elsewhere; so, again, the book is unnecessarily long despite having a useful summary of current work in this difficult area. The considerable interest of the biotechnology industry here, together with recent developments in handling and expressing human genes, will almost certainly ensure rapid development of this field.

Now that monoclonal antibodies are impinging on wide areas of biological and clinical science there is a great need for books to function as knowledge conversion kits similar to those available for upgrading construction toys for children. This would save readers considerable time and money. □

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Journey to adulthood

Leonard A. Freed

The Growth and Development of Birds. By Raymond J. O'Connor. *Wiley: 1984. Pp.315. £22, \$34.15.*

BIRDS are extremely varied with respect to the size and composition of their eggs, the nature of the nests they provide for eggs and neonates, and growth and development both during incubation and after hatching. They range from species without parental care following egg-laying to those in which care continues to long past the fledging stage.

Growth and development occur in: (i) a thermal environment influenced by nest, parental brooding, brood size, and maturation of individual endothermy; (ii) a nutritional environment influenced by diet and frequency of feedings; (iii) a social environment in which there is parent-offspring interaction, sibling rivalry for parental attention, and interaction with conspecifics during dispersal, migration and recruitment for breeding; and (iv) a demographic environment with risks of mortality at each life-history stage. The nature and combinations of these environments differ between taxonomic groups (indicating phylogenetic constraints on developmental patterns), as well as within them for species in different ecological circumstances (indicating evolutionary adaptation of developmental patterns).

Raymond O'Connor's *The Growth and Development of Birds* is an important first review of aspects of these environments through its attempt to combine physiological, behavioural and ecological studies

within an evolutionary perspective. The book is structured around the avian life-cycle, bringing together discussion of an unusually wide range of topics — including those mentioned above — and comparing altricial and precocial species for many of them. The goal of the book is clearly breadth rather than depth of coverage. But while specialists may be disappointed by the absence of certain details to do with their particular research interest, or the scarcity of work published after 1980, they will undoubtedly be stimulated by seeing their area put in context and perhaps moved to examine aspects of it they might previously have overlooked.

Readers should be aware that several topics (for example asynchronous hatching and optimization of growth rates) have become increasingly controversial since the book was written, and also that most examples are based on North Temperate species. Tropical birds, with different life histories and social structures, remain understudied in all aspects of growth and development. Nevertheless, the book will be read with profit by a wide range of biologists, and beginning graduate students and undergraduates will here encounter features of growth and development that they would be unlikely to come across in any single course. □

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• Academic Press has just published the third volume in the series *Form and Function in Birds* edited by A.S. King and J. McLelland, the emphasis in this volume is upon locomotion and the special sense organs. Price is \$99.50, £90

Out of space, out of time

John D. Barrow

Relativistic Astrophysics. By M. Demiański. Translated by A. Pol. Pergamon: 1985. Pp.341. £25, \$45.

RELATIVISTIC astrophysics means different things to different people. To some it is special relativistic astrophysics with an emphasis upon the physical complexities of high-energy radiation processes, pulsars, accretion disks and neutron stars; to others it means general relativistic astrophysics and the exotic realm of ultra-strong gravitational fields; black holes, gravitational collapse and gravitational waves. Marck Demiański's text is of the general relativistic sort.

The author assumes the student possesses a basic knowledge of general relativity and tensor calculus, the basics of which are completed by page four. The book then commences proper with a succinctly written study of black holes, including a real derivation of the Kerr solution via the Ernst potential and a study of its orbits; derivations of the laws of black hole mechanics and thermodynamics together with simple analyses of the stability of the Kerr and Schwarzschild black hole solutions come later.

Between these discussions of areas which have been of special interest to the author is sandwiched a less compelling section of relativistic hydro- and thermodynamics, with a heavy emphasis on for-

malism that in subsequently put to little use. But good chapters follow on the stability of isolated self-gravitating bodies in Newtonian and general relativistic gravity with a detailed discussion of the effects of rotation. There are less quantitative surveys of astrophysical processes in the vicinity of black holes and neutron stars but these fall short of the standards set by other recently published textbooks — in particular that of Shapiro and Teukolsky's *Black Holes, White Dwarfs and Neutron Stars* (Wiley: 1983).

The final chapter, devoted to cosmology, is very disappointing. Here, the fact that the text was first written in 1978 finally catches up with the publishers. Although the earlier chapters on mathematical aspects of relativistic astrophysics have not dated during the prolonged translation period, the subject of cosmology has been revolutionized to such an extent that the author's discussion of physical processes in the early universe and galaxy formation is seriously out of date. The most recent references in the bibliographies are to works published in 1978 and 1979.

As the starting point for a second course on general relativity and its non-cosmological applications this book can be recommended to students for its systematic and lucid exposition, but the reader who is interested in contemporary ideas in astrophysics and cosmology will find that the story told by Demiański has been rather overtaken by Old Father Time. □

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Going through the changes

Rita Cruise O'Brien

Stacking the Chips: Information Technology and the Distribution of Income. By J. Bessant and S. Cole. Frances Pinter, London/Rowman & Allanheld, 81 Adams Drive, Totowa, New Jersey: 1985. Pp.290. £17.50, \$26.95.

GETTING beyond the generalities and hyperbole in discussion of the effect of information technology on developing countries has been an uphill struggle. At a recent meeting of the North-South Roundtable (Society for International Development) on this topic, which included leading government spokesmen, scholars and business people, it became clear that some progress had been made. For some years the approach "Information Technology: Blessing or Plague of the Third World" prevailed making it difficult at times to make a sensible contribution.

Central to researching or writing about

informatics and development are questions of opportunity cost, technology transfer, income distribution and employment effects. I prefer to consider small corners within the field such as the experience of a single country or a particular problem which might be illustrative of others. For example, was it a wise government directive to have automated the Brazilian banking system, making banks more efficient at international dealing and trading, while destroying many jobs at the clerical level inside the country's banks? The authors of *Stacking the Chips* however, have opted for comprehensive analysis and refinement of economic modelling on the subject.

The authors did their work at the Sussex University Science Policy Research Unit (a centre of excellent research both on industrialized and developing countries) on a grant from the UN Institute for Training and Research. Ostensibly the book is about the effects of high technology on the distribution of income. The authors obviously felt it necessary to clear a lot of the ground first but instead of then delving more deeply into the subject, they introduce many different variables covering a

wide range of sectors. The analysis therefore in either scholarly or policy terms is rather unsatisfactory.

They provide a good introduction to the macro-economic literature on informatics and industrialization, employment and the comparative advantages, for example. But it ought to have been set out in a clearer more coherent manner. It is, for example, impossible to make quick reference to tables, which are not well labelled. The authors use a curious character coding which is difficult to follow because the key is to be found on Table 6.2, which is mentioned without giving a page reference. Books ought by first principles to be easily read and understood. And for students, hungry for material on the subject, the splendidly researched literature review is a hesitant and much qualified academic discussion rather than a clear indication of controversy or major contributions to knowledge.

Stacking the Chips works on a model developed by Cole in 1982 which demonstrated that technological change produced significant employment and distributional effects. This book, by its own description, attempts a very crude interpolation of available research evidence, despite Raphael Kaplinsky's warning in *Microelectronics and Employment Revisited* (ILO, Geneva, 1985) about the major gaps in coverage, methodology and analysis. After reading this latest contribution, I conclude that much indeed still needs to be done.

The conclusion that new technology does not necessarily lead to job destruction in the long term, but that shorter term structural problems are evident, repeats old caveats, many of which were pioneered at the Sussex University Science Policy Research Unit. With the exceptionally broad brief chosen by the authors, the obvious can only be reaffirmed. If in future they are at all interested in studies dealing with fewer technologies, sectors and a more modest terrain than the global economy in eight segments, I look forward to the sequel. □

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New editions

- *Ancient Sedimentary Environments (and their sub-surface diagnosis)* 3rd Edn, by R.C. Selley. Publisher is Chapman & Hall, price is hbk £27.50; pbk £10.95.
- *Color Measurement: Theme and Variations* 2nd Edn, by D.L. MacAdam. Publisher is Springer-Verlag, price is pbk DM 94.
- *Biotechnology: A New Industrial Revolution* revised edition, by Steve Prentis. Publisher is Orbis, London, price is £12.
- *Seismic Migration: Part A. Theoretical Aspects* 3rd Edn, by A.J. Berkhout. Publisher is Elsevier, price is Dfl.190, \$70.50.
- *Cryogenic Systems* 2nd Edn, by Randall F. Barron. Publisher is Oxford University Press, price is £60. This book may be available in the United States, but the publisher has been unable to provide that information.

Small-scale processes in the upper ocean boundary layer

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Here I review some of the recent advances in our knowledge of the physics of the upper ocean boundary layer at scales of micrometres to tens of metres, with emphasis on the nature of turbulence in the layer. A study of sub-surface bubbles produced by breaking wind waves offers some hope of revealing more of the turbulent processes, and indicates that bubbles may be important in the transfer of gases from air to sea.

OCEANOGRAPHERS are presently paying increased attention to the small-scale processes which operate at, or near, the sea surface. With rare exceptions, interest has been dormant since the exciting era in the 1950s when people such as Woodcock¹ were actively involved in seeking solutions to questions of air-sea interaction which required information about the small-scale physics, notably those relating to the production of spray and aerosols. As often happens, it has partly been the development of novel instruments which has revived this area of interest. The use of microwave satellite sensors—the synthetic aperture radar and scatterometer—and surface X-band radar has prompted new questions about the small-scale topography of the sea surface to which the instruments are sensitive. These questions must be answered if we are to make effective use of the measured data, perhaps to infer wind stress from the scatterometer by relations which are better than empirical, and hence improve the representation of atmospheric forcing in global models of ocean circulation. Better estimates of air-sea gas fluxes are required for global budget models and, for example, to enable oceanographers to use tracers such as tritium (with helium decay) to date water masses which lose contact with the surface and pass into—and ‘ventilate’—the main thermocline.

Some of the answers are already becoming available (in particular, studies² have been made of microwave scattering from breaking waves). Even the perennial questions of the role and importance of breaking waves in the transport of momentum from air to sea and of their generation of turbulence in the sea itself are close to being answered in a quantified way^{3,4}, although we still require a clear explanation of some puzzling but well-known phenomena, for example why it is that whitecapping is less frequent when it is raining than when it is not, other factors (for example, wind speed) remaining the same.

The fundamental question which remains to be answered is ‘What is the nature of turbulence below the sea surface?’ This

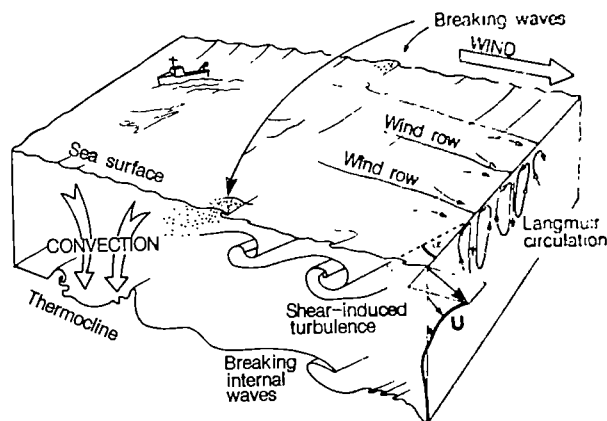
question concerns the ‘mixed layer’ or, to avoid ambiguity, the ‘upper ocean boundary layer’ (either term may include the seasonal thermocline). Some of the mechanisms affecting the turbulence are illustrated in Fig. 1. Moisture flux and radiative effects, essential in studies of convection, will be mentioned only in passing. I have also not discussed fronts; they are usually larger than the scales that I have chosen to describe.

Ocean/atmosphere similarities

It has frequently been assumed that the dynamics of the upper ocean boundary layer are similar to those of the atmospheric boundary layer. Indeed, studies of both mean quantities and fluctuations provide evidence in support of this hypothesis, although without exception the volume of data which is available for comparison is sparse and scarcely sufficient to confidently establish a similarity.

The mean flow in the upper few metres of the ocean and lakes has been measured by Churchill and Csanady⁵ in neutral conditions (those in which atmospheric heating or cooling is negligible). The near-logarithmic variation of speed with depth found by observing the motion of floats is similar to that of the wind in the atmosphere. Two of the authors’ conclusions are particularly remarkable. The ‘roughness length’ in the water (a length scale found empirically from the observations and, as the name implies, some measure of the nature of the surface) is some two orders of magnitude greater than that in the air (of the order of tens of centimetres rather than a few millimetres). Second, in conditions when the wave field was strongly affected by swell, much higher values of friction velocity, u_* (equal to the square root of the stress divided by the fluid density), in the water were inferred than could be explained by a conventional wind-drag relationship. The authors suggested, however, that this might be accounted for by the greater importance in these conditions of the wave-induced mean drift in the water—the

Fig. 1 Some of the mechanisms affecting turbulence in the upper ocean boundary layer. The spiral-like structure of the mean current (U) is due to the Earth’s rotation and reverses in sense in the Southern Hemisphere. The orientation of the ‘billows’ labelled ‘shear-induced turbulence’ is determined by the mean shear, so that their (horizontal) axes are not at right angles to the wind (that is, on average, angle $\alpha > 0$ in the Northern Hemisphere). Breaking surface waves and Langmuir circulation are, in particular, processes responsible for redistributing floating particles or biota. Breaking internal waves are included in the diagram, although they are not discussed in the text; they may be important in affecting the flux of nutrients and occasionally have an effect at the sea surface itself (see ref. 41). The wakes of ships will rarely be significant contributors to the net fluxes, except with respect to low-frequency sound!



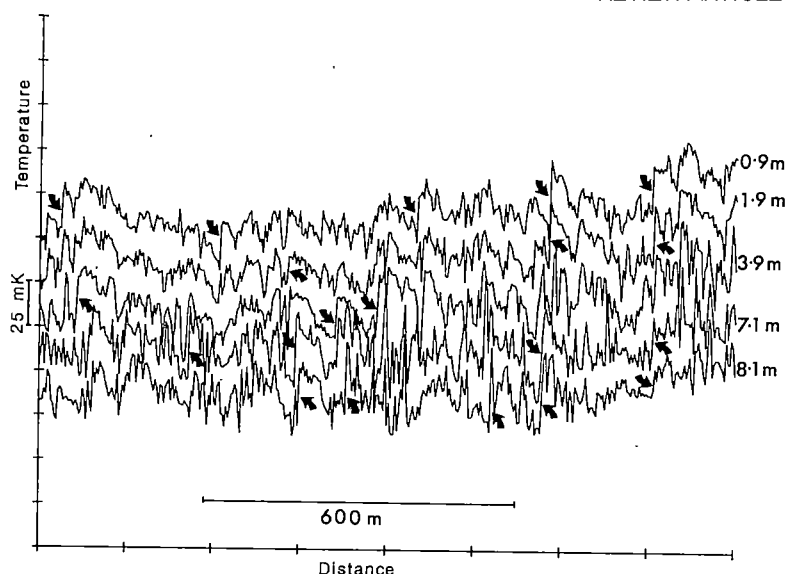


Fig. 2 Temperature fluctuations measured at the indicated depths from a spar hanging beneath a towed catamaran near 38°40'N, 12°50'W in October 1984 in daytime under clear skies. The wind speed was $\sim 7 \text{ m s}^{-1}$. Time variations have been converted to distance using the mean towing speed through the water, 1.9 m s^{-1} . Some of the abrupt temperature rises are marked by arrows.

'Stokes drift'. (The use of floats, or 'drifters', to measure drift currents may, however, be less than ideal because of the higher than average horizontal currents found below wind rows towards which floating objects tend to be drawn; I shall address Langmuir circulation below.) Flows in non-neutral conditions have yet to be closely studied in the ocean. However, it is known that the diurnal heating cycle can have a profound effect in modulating the oceanic near-surface stratification and currents, just as it does in the atmosphere⁶. A diurnal modulation of the current in the near-surface ocean, a variation resembling the well-known nocturnal jet in the atmosphere⁷ but being essentially a daytime event, has been observed⁸ and modelled^{8,9}; it results from the daily cycling of the layer depth into which wind-driven momentum is distributed as the stratification varies due to solar heating, and the subsequent inertial development of the current under the effect of the Earth's rotation.

Turbulence in the atmosphere is driven by convection and by the stress of the wind on the underlying boundary. In the ocean, turbulence may be similarly driven by convection and by wind stress, although less directly as waves act as an intermediary (see below). In the case of turbulent motions in near-neutral conditions, a similarity of the horizontal velocity spectra in lakes and ocean has been found¹⁰ when they are scaled with depth, z , and friction velocity. The spectra collapse into the canonical spectral form of flow in a turbulent boundary layer, as determined by laboratory experiments (ref. 11; see also ref. 12). The scaling of the rate of dissipation of turbulent kinetic energy in the ocean, ϵ , with the cube of the wind speed¹³ and inversely¹⁴ with depth provides more support for constant stress layer similarity, but these are relationships which deserve further investigation. Such investigation is now possible through the development (notably by Dillon; private communication) of upward-profiling turbulence probes capable of measurements right up to the sea surface. The most convincing demonstration of similarity between the oceanic and atmospheric boundary layers is perhaps the study of convective conditions in the ocean¹⁵ and the comparison with atmospheric data¹⁶. It is found that in both the ocean and the atmosphere, ϵ is uniform throughout much of the layer where convection dominates over the effects of surface stress (that is, beyond a Monin-Obukov length from the surface) and is proportional to the effective flux of buoyancy due to the heat passing through the surface. It is only near the surface that differences between the atmosphere and ocean appear significant (that is, $z/D < 0.3$ in the comparison of ref. 15, where D is the depth to which convection extends, here $\sim 150 \text{ m}$). The turbulence probes used in that study are now being used to address fundamental questions about the turbulent structure of stratified fluids and to obtain the measurements of turbulence and diffusion in the upper ocean that are

required for numerical models of the ocean (see, for example, ref. 17).

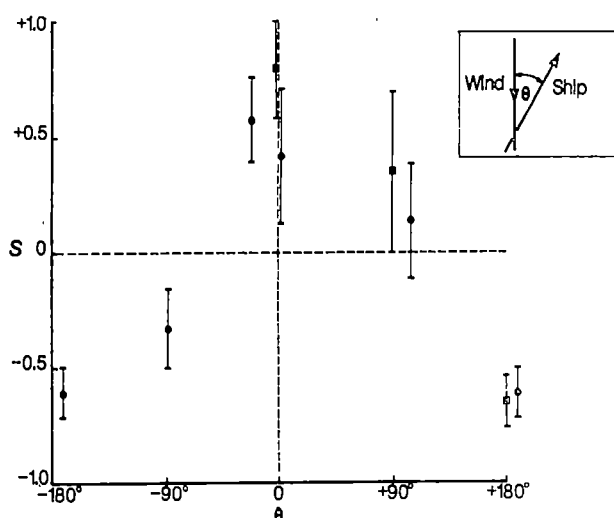
One other measurement in which similarity is found between ocean and atmosphere is that of temperature structure. Temperatures measured at fixed positions in the atmospheric boundary layer are found to have a 'sawtooth' pattern, with rather abrupt changes (predominantly in the same sense in given conditions of heat flux) being followed by relatively gradual ramps, thus leading to skewed temperature derivatives¹⁸. Similar skewed gradients have been reported from towed or moored instruments in a lake¹⁹, and have now been observed at sea (Figs 2, 3). In conditions in which the heat flux leads to stable stratification, the skewness measured in upwind tows (or from moorings) is positive; it reverses sign in downwind tows. Intriguingly, the skewness usually appears to be slightly positive in tows made to the right of the wind ($\theta = 90^\circ$ in Fig. 3; this is reversed in tows to the left of the wind), presumably because the Ekman flow, the rotation of the current vector with depth due to Coriolis forces, leads to a pattern of eddies or 'billows' which are, on average, rotated as sketched in Fig. 1 (the features labelled 'shear-induced turbulence'). If so, the trend should reverse in the Southern Hemisphere. It has not yet been established that inverse temperature ramps and skewness of the opposite sign are found in convectively unstable conditions in the ocean, but this may be expected from experiments in the laboratory and in the atmospheric boundary layer²⁰. Specifically, the sign of the skewness of the streamwise temperature gradient, sign S , is given by sign $(\mathbf{x} \cdot \Delta T \times \boldsymbol{\omega})$, where \mathbf{x} is a vector in the flow direction, ΔT is the gradient of the mean temperature and $\boldsymbol{\omega}$ is the vorticity of the mean flow.

The existence of temperature ramps—and these have some coherence in the vertical (see Fig. 2)—suggests the presence of organized structures²¹; this is not surprising as large coherent structures are now known to be a feature of turbulent shear flows. We need, however, to discover whether significant vertical fluxes of heat and momentum are carried by these structures in the ocean for, if this is the case, it might be possible to devise a semi-deterministic model of the turbulence by specifically accounting for their presence. The construction of such a model is feasible, especially if, as is possible, the flow is close to marginal stability in conditions of stable heating^{22,23} and the ramps are related to the most 'dangerous', or—once instability occurs—most rapidly growing modes.

Langmuir circulation

This brings me to comment briefly, but emphatically, on the near-surface organized structure consisting of alternating vortices aligned downwind which are known as the Langmuir circulation. This is well known to produce a coherent structure

Fig. 3 Variation of the skewness of the temperature derivative, S , measured in tows at constant depth at different angles, θ , to the wind when the net air-water heat flux is positive. The circles represent measurements made at sea (for example, in the Northern Hemisphere, as in Fig. 2) and the squares represent measurements from Loch Ness¹⁹. Corresponding values of S at $\theta=0$ in the atmospheric boundary layer are in the range 0.4–0.85 (refs 18, 20).



at the sea surface—the associated wind rows into which floating material is carried—and, as Leibovich²⁴ has suggested, we might also account for its effects on the fluxes below the surface in a deterministic way.

It is a profound embarrassment to physical oceanographers that we have not yet established whether, or when, Langmuir circulation is important in distributing heat and momentum in the upper ocean or in forming the seasonal thermocline. This has remained one of the most challenging but well-defined tasks in physical oceanography since Langmuir wrote his definitive paper 47 yrs ago⁴². Recent observations made from the ocean research platform Flip²⁵ have demonstrated that, in addition to the enhanced downwind velocities which I mentioned above, remarkably large downward velocities can be measured beneath wind rows. The vertical momentum carried by these circulations—the associated Reynolds stress—appears too great in some of the cases studied to be in equilibrium with the local wind, suggesting a considerable variability in structure; more observations are required to link measurement to theory. Whether the circulation has an immediate counterpart in the atmospheric boundary layer depends on how it is generated or on which theory one accepts. The most plausible theory (in that it best satisfies the existing observations) is that development by Craik and Leibovich²⁴. The pattern of streamwise vortices is generated by the interaction of waves and mean shear. Some of the theoretical predictions have been verified in the laboratory²⁶. We know the mechanism works, but is it the source of circulation in the ocean, and can theory be used to predict the associated fluxes?

Breaking waves

The differences between oceanic and atmospheric boundary layer dynamics are due partly to radiative and evaporative processes, but also to the asymmetrical nature of the sea surface, the turbulence generated by breaking waves, and the relative importance of mean flow, wave-induced motions and turbulent fluctuations. A fundamental asymmetry is found in the shape of surface waves; they have narrow crests and relatively broad troughs. The formidable nonlinearity of the boundary condition at the free surface makes the theoretical study of wave instability and of the onset of breaking one of great complexity, yet considerable advances have been made (see, for example, ref. 27). It has long been known that breaking occurs at or near the wave crests; this results in the production of sub-surface bubbles and of foam sometimes compacted into a layer many bubbles deep. Spray, the antithesis of bubbles, is thrown into the air, but whilst the opposite of foam—drops of water surrounded by air but resting on the interface—does occur briefly, the drops are very unstable and do not build up into compact layers. It is almost frivolous to mention that rarely is there in the ocean a deep source of rising bubbles; the antithesis of rain is rare. One

particular feature pertinent to the role of bubbles in contributing to the gas flux across the sea surface is that they have only to be carried down to a depth of ~ 10 m before their internal pressure is doubled hydrostatically. Spray, on the other hand, has to ascend to over 5 km before it experiences a reduction in pressure to a half of that at its origin at the sea surface. The wind speed at 10 m above the sea surface, W , is usually comparable to the dominant wave speed, c . The turbulent and wave-induced fluctuations in the air are comparable in magnitude, but are generally less than the wind speed. In the near-surface ocean, however, the wave speed and the wave particle velocities usually greatly exceed the mean flow and the turbulent fluctuations. Motion is dominated by waves. The critical layer where the wave speed matches the mean flow occurs in the air rather than in the water. (The water, however, generally carries the greater momentum per unit volume, and has a much greater heat capacity.)

The development of multiply connected surfaces as waves overturn and fluid re-enters through the water surface, allows the waves to generate vorticity (a characteristic of turbulent motion) even in a pre-existing irrotational flow; the conditions (described by Kelvin's circulation theorem) in which vorticity is conserved are violated. There are several possible candidates responsible for producing turbulence in breaking waves, perhaps the most obvious of which is instability at the head of, and around, the injection jet at the tip of a plunging wave after its entry into the water surface. A second candidate is the wake region of large rising bubbles, especially spherical-cap bubbles, entrained by a breaking wave. But a more potent and frequent source in both plunging and spilling breakers appears to be the shear layer formed between the spilling water of the breaker zone moving at about the speed of the wave crest and the underlying water moving backwards relative to the crest. The instability of this shear zone may be the source of oscillations in the spilling zone of breaking waves observed by Banner and Fooks².

Very little is known of the nature (for example, spectra and patchiness) of wave-induced turbulence, although this is becoming an active area of research. Some recent advances, especially those in laboratory experiments, are reviewed in the proceedings of a conference held recently in Japan²⁸. Most of the measurements of velocity fluctuations very near the sea surface have been concerned primarily with those at, or near, the wave frequencies. Early measurements at higher frequencies²⁹ concluded that almost all the turbulent energy produced by waves must be dissipated in the upper few metres, whilst Russian workers³⁰ found that the near-surface Reynolds stress decreased with depth and appeared to be associated with wave frequencies. Kitaigorodskii *et al.*³¹ have recently analysed measurements of turbulence made with a drag sphere mounted on a lake tower, and conclude that there is intense wave-generated turbulence

in the region immediately below the surface, of thickness about 10 times the mean wave amplitude, ζ , whilst at greater depths the turbulence has the character of the conventional constant stress layer. The region of wave-generated turbulence is quite thick: values of ζ of 2 m, which is not uncommon, imply that the region extends to 20 m below the surface! Further work is required to substantiate these observations.

Bubbles and turbulence

Clouds of sub-surface bubbles produced by breaking wind waves can be detected by narrow-beam upward-pointing sonars moored or mounted on the sea bed^{32,33}. The mean depth to which these clouds of bubbles extend, \bar{d} , non-dimensionalized with wavelength, λ , increases with the ratio of wind to wave speed, W/c ; typically, in open sea conditions, \bar{d} reaches 10 m for winds of $\sim 14 \text{ m s}^{-1}$. The acoustic scattering cross-section of the bubble clouds at a fixed depth, M_v , a measure of the approximate area of the bubbles per unit volume when the sonar frequency is sufficiently high, increases rapidly with wind speed (roughly as W^{12}) but decreases roughly exponentially with depth with a decay scale of 0.5–2 m. In high winds the status layer of bubbles formed below the surface can be dense enough to muffle the ambient noise in the sea produced by breaking waves³⁴.

The challenge is to solve the inverse problem—to obtain information about the turbulence from the measured vertical distribution of the bubble clouds; this has been attempted by comparing the observed distribution with predictions from models based on a balance between turbulent diffusion, the upward buoyant rise of bubbles of a given radius, and the flux in radius space due to bubbles being compressed hydrostatically as they are carried downwards or by their exchanging their constituent gasses with those dissolved in the water. Several assumptions made in the models raise further interesting questions about the turbulent dynamics near the sea surface and the analogies between water droplets in the atmosphere and bubbles in the sea. Various representations of turbulence have been tried³⁵, the most successful being that of adopting an eddy diffusion coefficient, K_v , of the classical boundary layer form, ku_*z , where k is Von Karman's constant. The values of u_* required for agreement with the observations are, however, greater than those derived from the wind speed, assuming a typical drag coefficient and continuity of stress across the sea surface. The ratio is close to unity for W/c near 2, but increases to ~ 2.5 for $W/c = 0.8$, when swell is of greater importance in the wave field. This result, reminiscent of Churchill and Csanady's⁵ conclusions based on drift currents, deserves further critical and careful investigation. A fit using a form of K_v which tends to a constant value in the surface layer but tends to the conventional ku_*z at depth, and which assumes that u_* is given by the wind drag, gives a mean surface layer thickness of 9.7 ζ ,

which is intriguingly close to the conclusion of Kitaigorodskii *et al.*³¹ concerning the region dominated by wave turbulence.

The temporal variation of bubble clouds at a fixed position (and this is what is measured by a moored sonar) can be linked via advection and the lifetime of bubble clouds to the spatial and temporal distribution of the breaking waves which create them, and so to the whitecap area. Here there is the prospect of linking bubbles, turbulence, waves and foam to the questions of momentum transfer³⁶. We are again moving towards more deterministic models of forcing the turbulence in the sea; momentum is injected in pulses when wave groups break at a rate just in balance with the wind-forced growth of waves between breaking events^{3,4}.

Gas transfer

I must finally mention gas transfer because this is another area of upper ocean research in which significant advances are being made. Kitaigorodskii³⁷ has directed attention to the importance of patches of enhanced turbulence generated by breaking waves in changing the thickness of the viscous sublayer at the surface across which gases are exchanged with the atmosphere. Others^{33,38,39} have pointed to the possible importance of bubbles. As bubbles are carried beneath the surface, their internal pressure increases considerably and this acts like a piston to drive gas into the surrounding water, at least until the water itself becomes supersaturated. The equilibrium condition is thus one in which the water is supersaturated, with a balance being achieved between the input of gases via the bubbles and the evasion of gas through the sea surface. (Here is another feature of the asymmetry of the air-sea interface.) The bubble production increases with wind speed and so does the level of supersaturation at which equilibrium occurs. Present measurements suggest that in typical wave conditions, a mean wind speed of $\sim 8 \text{ m s}^{-1}$ may be sufficient to support a 3% supersaturation level in the water, about the average reported for oxygen in the Pacific⁴⁰. However, as M_v (and hence bubble area) increases rapidly with wind speed, an occasional storm may be sufficient to maintain enhanced levels of supersaturation equivalent to those produced by other processes (for example, biological).

Conclusion

We are slowly approaching improved, better determined and less empirical models of the upper ocean boundary layer through attention to small-scale processes. To be properly interpreted, these processes demand an understanding of the physics from scales upwards of a few micrometres, the size of the smallest bubbles. The processes are essential elements in the exchanges between the atmosphere and the ocean, and advances in understanding them will lead to improved estimates of the global budgets of energy, heat and gases.

Mr Alan Hall provided the data shown in Fig. 2.

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Solar signature in sedimentary cycles from the late Precambrian Elatina Formation, Australia

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Climatic cyclicity is recorded by regular variations in the thickness of varves in the Elatina Formation, a late Precambrian periglacial lake deposit in South Australia. Earlier conclusions that the climatic cycles reflect solar variability are strongly supported by a complexity of periods revealed through study of drill cores of the varved sequence. The new data promise to contribute significantly to our understanding of solar processes.

APPARENT solar periods (principally ~11 and ~22 yr) were previously recognized^{1,2} in thickness variations of varved (annually layered) periglacial lake deposits of the late Precambrian Elatina Formation in the Flinders Ranges, South Australia. Because the rocks are not well exposed, diamond drilling was carried out in December 1982 to obtain a complete cored sequence of the ~10-m-thick varved interval. Some drill core was lost through rock fracturing, so a group of three vertical holes was drilled to allow matching of cores and construction of the longest possible continuous varved sequence. Logging the drill cores and correlation of the three sequences³ produced a stratigraphically continuous log, 9.39 m long and spanning an estimated ~19,000 yr VT (varve time), which could be measured precisely.

The new data reviewed here support the original conclusion that solar periods are recorded by the rocks, and may prove invaluable to our understanding of the solar activity cycle.

Geological setting

The Elatina Formation at Pichi Richi Pass in the Flinders Ranges, South Australia, comprises siltstones and fine sandstones deposited in a periglacial lake during the Marinoan Glaciation ~680 Myr ago¹⁻⁴. The occurrence of sand wedge structures in a penecontemporaneous fossil permafrost horizon within the adjacent palaeo-catchment area provides good evidence of a markedly seasonal, arid Marinoan periglacial climate⁴ and implies strong seasonal control on meltwater discharge into the periglacial lake.

The laminated interval of the Elatina Formation records climatic cyclicity through regular variations in the thickness and grain size of graded (fining-upward) laminae up to 3 mm thick (Fig. 1) that were deposited by density underflows in the distal portion of the lake^{1,2}. Scattered 'drop grains' up to the size of small pebbles were probably derived from melting river ice. The interval is characterized by conspicuous groups or cycles of about 10-14 laminae that are usually bounded by thinner, darker, more clayey laminae. The thickness of laminae varies systematically within each cycle, attaining a maximum near the cycle centre. A cycle 'doublet' of alternate thick and thin cycles is common (Fig. 1) and longer rhythms are evident through regular changes in cycle thickness.

The non-random variation in thickness of the graded laminae, together with the envisaged palaeogeographical setting and strongly seasonal arid palaeoclimate, provide good evidence that the laminae record non-random spring or summer meltwater discharge into the lake and that each lamina is, therefore, an annual increment or varve. The laminae are similar to the 'intermediate distal facies' in the varve classification of Smith⁵, which comprises delicate but distinct varves with summer layers usually too thin to display sublaminae. Through comparison with modern glaciolacustrine sedimentation⁶, varve thickness is interpreted as a measure of relative mean annual temperature. Plots

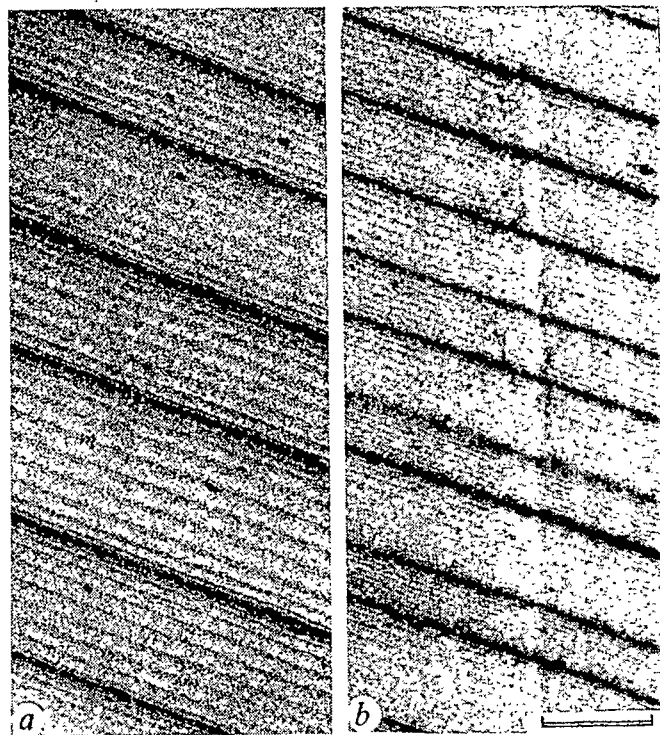


Fig. 1 Cyclic lamination in drill core from the Elatina Formation, Pichi Richi Pass. Conspicuous cycles comprise groups of ~10-14 graded laminae of silt to very fine sand grade, bounded by thinner, darker, more clayey laminae. The lower half of *b* displays cycle 'doublets' resulting from alternate thick and thin successive cycles. Scale bar, 1 cm.

of varve and varve-cycle thickness against time^{1,2} therefore may also be viewed as plots of time-variation in relative mean annual temperature. As the thinnest varves reflect the coldest portion of the climatic spectrum, their more clayey composition may be attributed to deposition of suspended fines through more widespread freezing or major overturn of cold surface waters¹. The scattered distribution of 'drop grains' throughout the cycles, with no preferred concentration in particular laminae, suggests, nonetheless, that significant pauses in annual density underflow events did not occur near varve-thickness minima.

Time-series analysis

From thickness measurements of drill-core material, distinct periodicities have been identified in two related sequences: (1) a detailed sequence of 1,337 varves obtained from enlarged photographs of thin-sections; (2) a long sequence of 1,580 cycles, each cycle comprising from 8 to 16 varves, obtained from the composite log of the drill cores.

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Table 1 Data for conspicuous cyclicity in the Elatina time series

<i>a</i> Defined by varve thickness				
	Mean period (yr VT)	s.d. (yr VT)	Range (yr VT)	Repetitions
Measured between minima	12.0	1.6	8–16	110
Measured between maxima	12.0	1.8	8–16	110
<i>b</i> Defined by maxima in ~12-yr VT cycle thickness ('Elatina cycle')				
	Mean period (~12-yr VT cycles)	s.d.	Range	Repetitions
Unsmoothed curve (Fig. 3a)	26.2	1.5	20–29	59
Smoothed curve (Fig. 3b)	26.2	0.9	23–28	59

Table 2 Main periods identified in the Elatina time series

Visual mean period	DFT Period	PSD*	Visual mean period	DFT Period	PSD*
Detailed sequence (yr VT)			Long sequence (~12-yr VT cycles)		
	8.0	0.03		2.1	0.51
	9.7	0.06		3.8	0.01†
	10.3	0.29		4.4	0.02†
	11.0	0.46		5.3	0.04
	11.2	0.60		6.6	0.07
	11.6	0.23		8.8	0.16
12.0	11.9	0.66	13.1	13.1	0.55
	12.3	0.54	26.2	26.1	1.00
	12.5	0.64			
	13.0	0.30	Long sequence, low frequency ³ (~12-yr VT cycles)		
	13.5	0.24			
	14.4	0.16		134	0.21
	22.4	0.18		195	0.19
	25.8	0.21	700–800	778	1.00
	103.2	0.15			
~156	158.6	0.45			
~312	333.2	1.00			

* Power spectral densities (PSD) normalized to unity for the strongest periods.

† PSD from DFT of 'high-frequency' curve (see Fig. 3c).

Thicknesses were determined with a precision of 0.01 mm at the Laboratory of Tree-Ring Research and recorded on a floppy disk; the data were then transferred to the Lunar and Planetary Laboratory's INTERDATA 8/32 computer for time-series analysis.

Detailed sequence. Well-preserved varves contained in a piece of core 93 cm long were measured from photographic enlargements (4.4×) of overlapping thin sections³. In places very thin varves, possible random (intra-annual) layers¹, and rare soft-sediment deformation made counting uncertain. Such places were few, however, and indicate an accuracy of ±5% for the count of 1,337 measurements.

The 1,337 varves span 110 complete cycles near the top of the core sequence of 1,580 cycles (cycles 1,405–1,514). Conspicuous fluctuations in varve thickness (Fig. 2) define a cycle ranging from 8 to 16 yr VT in duration and with a mean period of 12.0 yr VT (Table 1a). The cycles show an overall tendency to positive skewness, the mean ascent period being 5.57 yr VT and the mean descent period 6.45 yr VT. Characteristically, varve thicknesses fall to approximately the same minimum level every ~12 yr VT (mean = 0.19 mm, s.d. = 0.07 mm), whereas the intervening maxima have a large range in thickness (mean = 1.20 mm, s.d. = 0.47 mm). Other features include the occurrence of multiple peaks for certain maxima (Fig. 2a), the very minor 'spikes' within some ~12-yr VT minima (Fig. 2a, b), and the common

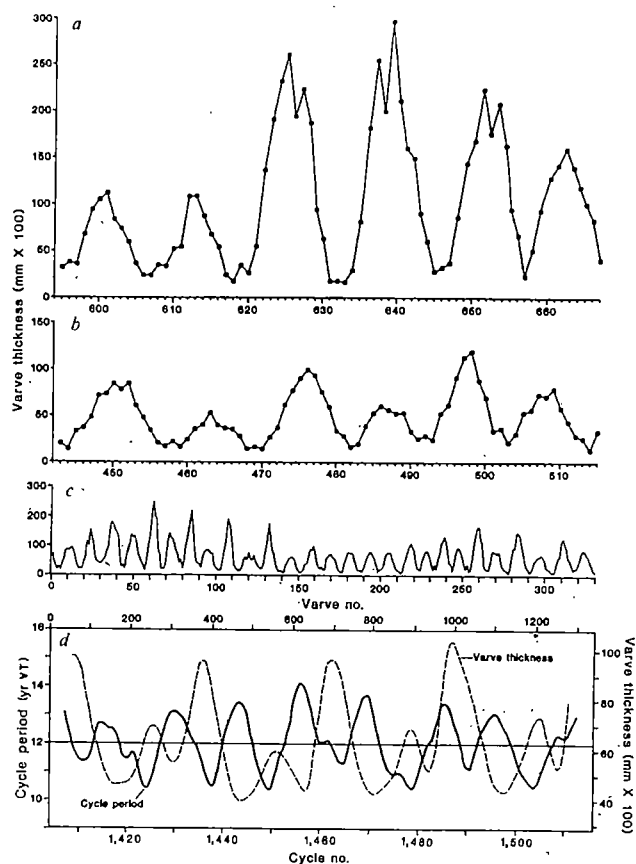


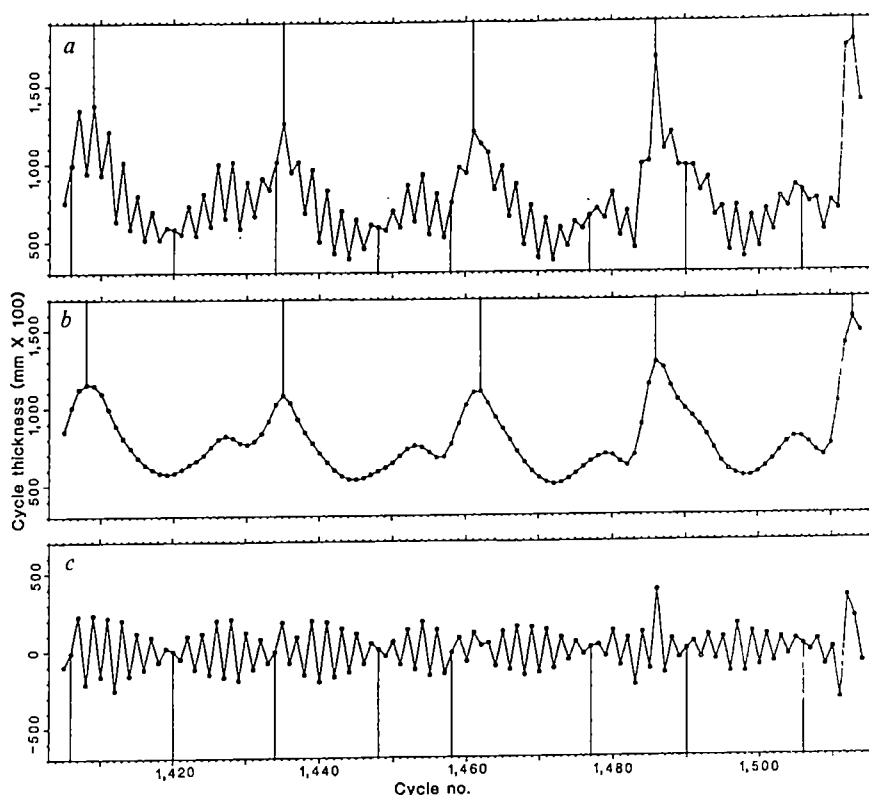
Fig. 2 Thicknesses of varves, extracted from the detailed sequence. (True thicknesses shown, obtained by dividing measurements from photographic images by 4.4.) Varve number increases up-sequence. *a*, Thick varves producing high-amplitude cycles with multiple peaks. A minor 'spike' occurs within a thickness minimum at varve 620. *b*, Thin varves producing low-amplitude cycles that display alternate higher and lower maxima. *c*, Small-scale plot showing long-term modulation of varve-cycle amplitude. *d*, Varve thickness for the entire detailed sequence (smoothed by 111-point filter), and variation with time in the period of the ~12-yr VT cycle measured between thickness minima (smoothed by five-point filter) for the same stratigraphical interval.

occurrence of alternating cycles of high and low maxima (Fig. 2b, c). In addition, regular fluctuations in the amplitude of the ~12-yr VT cycles and in varve thickness define longer periods of up to ~26 cycles (Fig. 2c, d).

The smoothed plot of cycle period against time (Fig. 2d) indicates that the long-term variation in cycle period is non-random; cycle periods are modulated by a longer rhythm with a mean period of about 13 cycles. Figure 2d shows that the thickest varves tend to coincide with relatively short cycle periods. Such negative correlation means that stronger ('higher') varve cycles tend to be of shorter duration than average. (This explains the early estimate of ~11 yr VT for the mean cycle period^{1,2}, as outcrop specimens displaying thicker, more easily counted varves and cycles were selected for examination first.) **Long sequence.** The excellent correlations obtained among the three drill holes indicate that a high level of confidence can be placed in the composite log of 1,580 ~12-yr VT cycles. Figure 3 shows the plot of cycle thickness against time for the interval of 110 cycles covered by the varve-thickness measurements, which is representative of the full sequence of 1,580 cycles.

Maxima in ~12-yr VT-cycle thicknesses occur on average every 26.2 cycles or ~314 yr VT (Table 1b). This long cycle, termed here the 'Elatina cycle', is repeated 59 times in the measured sequence. Second-order peaks that occur consistently in the same position relative to the maxima of the Elatina cycle

Fig. 3 Thicknesses of 110 ~12-yr VT cycles, extracted from the long sequence. Cycle number increases up-sequence. *a*, Cycle thicknesses showing distinct maxima every 25–27 cycles and a sawtooth pattern reflecting the characteristic alternation of relatively thick and thin cycles. *b*, Five-point smooth of the curve shown in *a*. *c*, High-frequency curve obtained by subtracting curve *b* from curve *a*. The vertical lines above the curves (*a*, *b*) mark boundaries of Elatina cycles and below the curves (*a*, *c*) positions of 180° phase reversals.



(Fig. 3b) confirm the presence of a second harmonic^{1,2} with a mean period near 13.1 ~12-yr VT cycles (~157 yr VT).

Superimposed on the Elatina cycle is a sawtooth pattern resulting from the alteration of relatively thick and thin ~12-yr VT cycles (Fig. 1b). The high frequency curve (Fig. 3c) reveals that the sawtooth pattern comprises a succession of envelopes displaying 180° phase reversals at or near the 'necks' between envelopes. A total of 106 such phase reversals occurs in the measured sequence; the interval between reversals averages 14.6 ~12-yr VT cycles (s.d. = 2.2, range = 9–23). The phase reversal is evidently a quasi-periodic function and no long-term modulation of the interval between reversals is apparent. The overall sawtooth pattern with phase reversals is described mathematically as a beat between two oscillations with periods near two cycles. As the mean period for a 360° change of phase (29.2 ~12-yr VT cycles or ~350 yr VT) is longer than the mean period of the Elatina cycle (26.2 cycles), the positions of phase reversals gradually advance up-sequence relative to amplitude maxima. The structure of amplitude maxima, therefore, varies according to their position relative to that of envelopes and necks of the sawtooth curve (Fig. 3a). As any miscount would break the observed pattern of phase reversals, the persistence of the pattern confirms the accuracy of the long sequence.

The Elatina Formation also records very long periods, notably a low-amplitude oscillation of period roughly 700–800 ~12-yr VT cycles discernible in the full plot of ~12-yr VT cycle thicknesses³.

Spectral analysis. The time series discussed above were analysed in several complementary ways: discrete Fourier transform (DFT), maximum entropy and least squares. All three methods gave similar results with regard to periods identified, although only the DFT results are discussed here. The periods determined by spectral analysis confirm those previously identified visually (Table 2), and are divisible into periods <30 yr VT, the Elatina cycle and its harmonics, and very long periods.

The DFT spectrum of the 1,337 varve-thickness measurements (Fig. 4a) reveals several discrete periods in the range of 10–14 and 22–25 yr VT, as well as longer periods of ~103, ~158 and ~333 yr VT. Additional, weaker peaks not shown in Fig. 4a occur at 8.0 and 9.7 yr VT. A beat between the periods identified at 22.4 and 25.8 yr VT may account for the beat pattern in the long sequence (Fig. 3c).

The DFT spectrum of the 1,580 ~12-yr VT cycles (Fig. 4b) reveals a striking 'harmonic series' comprising a fundamental period of 26.1 cycles and progressively higher harmonics of 13.1, 8.8, 6.6 and 5.3 cycles. Spectral plots at more detailed scales than Fig. 4b reveal two further harmonics at 4.4 and 3.8 cycles. The strong period near 2 cycles represents the ubiquitous cycle doublet. Good agreement exists between mutual periods identified in the detailed and long sequences, although the period of 26.1 cycles (Fig. 4b) is better resolved than the equivalent period of ~333 yr VT (Fig. 4a), which is beyond the limit of accurate resolution for the detailed sequence. Indeed, the consistency between data for the two sequences argues for the overall accuracy of the detailed sequence.

The Elatina cycle comprises, in accord with Fourier series expansion, a fundamental frequency and a number of higher harmonics (in the present case, six) of that fundamental. The DFT power spectral density amplitudes (*A*) of the Fourier series coefficients define an exponential function which is approximated by the equation

$$A \approx 220 \exp(-0.8f)$$

where *f* is the harmonic number (C. J. Durrant, personal communication). The harmonic series is also represented in the spectra of the detailed sequence (Fig. 4a).

DFT of the low-frequency portion of the long sequence suggests very long periods around 134, 195 and 778 ~12-yr VT cycles (~1,600, ~2,340 and ~9,330 yr VT respectively). The longest period is consistent with the low-amplitude oscillation discernible in the data³ but, statistically, is of uncertain significance in view of the relatively short record analysed.

As noted earlier, the period of the ~12-yr VT cycle appears to be modulated by a period near 13 cycles (Fig. 2d). DFT of cycle period versus time indicates that the period of the ~12-yr VT cycle is modulated principally by a period of 13.6 ~12-yr VT cycles (relative power spectral density = 1.00) and to a lesser degree by periods of 2.2 (0.73), 3.7 (0.18) and 27.8 (0.18) cycles (the latter period is, however, beyond the limit of accurate resolution). The periods of about 27.8 and 13.6 ~12-yr VT cycles may be equated respectively with the fundamental and second harmonic of the 'harmonic series' in the amplitude spectrum. Although the fundamental harmonic predominates in modulating ~12-yr VT-cycle amplitude, it is the second harmonic that

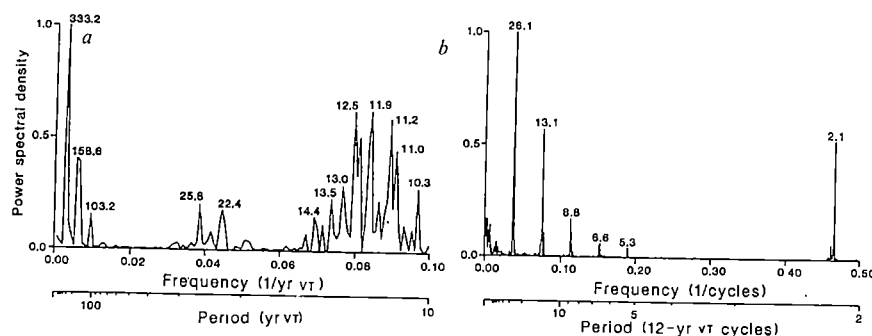


Fig. 4 DFT spectra normalized to unity for the strongest lines. *a*, DFT of 1,337 varve-thickness measurements. Spectrum unsmoothed. Linear frequency scale. *b*, DFT of thickness measurements of 1,580 ~12-yr VT cycles. Spectrum unsmoothed. Linear frequency scale. Peaks for the first to fifth harmonic of the ($26 \times \sim 12$ -yr VT) Elatina cycle are discernible.

predominates in modulating cycle frequency. The period near 2 cycles reflects a tendency for cycles of short duration to alternate with cycles of long duration, although such alternation in period shows no systematic relation to the amplitude alternation of the ~12-yr VT cycle (Fig. 3c).

Discussion

The periods recorded by the Elatina Formation cannot be explained by endogenetic processes within a glaciolacustrine system¹. Similar cyclicity has now been observed in penecontemporaneous lacustrine deposits up to 400 km from Pichi Richi Pass, indicating widespread climatic control of sedimentation. The new data generated by study of the drill cores support earlier conclusions^{1,2}, based on the study of limited outcrop, that the recorded climatic periods ultimately reflect solar variability.

The Elatina periods of 10–14, 22–25 and ~105 yr VT may be equated with cycles in the sunspot record, which similarly comprise several discrete periods between 8 and 13 yr, the Hale cycle of ~22 yr, and a longer period between 90 and 110 yr (refs 7–9). The longer Elatina periods of ~157 and ~314 yr VT are comparable to solar and climatic periods for Holocene time as indicated by tree-ring studies^{2,10,11}. The very long periods of up to ~9,300 yr VT may reflect very long-term climatic oscillations; possible counterparts exist in the recent climatic record^{12,13}, although a solar connection for such periods is not established.

The plot of varve thickness against time (Fig. 2) is comparable to that of mean annual sunspot number since AD 1700, particularly the reliable record since about AD 1830. Cycles in both the varve and sunspot series display a wide range of periods (8–16 yr VT for the Elatina Formation, 9.0–13.6 yr between minima and 7.3–17.1 yr between maxima for sunspot cycles¹⁴). The structure of cycles in each series is also comparable: minima fall to approximately the same level whereas maxima display a wide range of values; alternating cycles of high and low maxima are common; and varve cycles display an overall tendency to positive skewness, a feature also displayed by sunspot cycles^{15,16}. Furthermore, some varve-cycle maxima have multiple peaks, and minor 'spikes' occur within some minima; comparable features are displayed by the plot of mean annual sunspot (umbral) areas since AD 1920 (ref. 15). The similarity of structure of varve and solar cycles argues for a direct connection between varve thickness and solar activity, a conclusion reached previously on the evidence of overall skewness of a limited sample of varve cycles and the close similarity of portions of the varve-

cycle thickness curve to the plot of annual mean sunspot number at maxima^{1,2}. Varve-cycle structure would be quite dissimilar for an inverse relation between varve thickness and sunspot number.

The frequency modulation of the ~12-yr VT cycle (Fig. 2d) militates against the graded laminae representing monthly tidal increments, as such modulation is incompatible with regular tidal periods; indeed, the Elatina periodicities afford no evidence of tidal influence on sedimentation. Interestingly, the duration of the sunspot cycle over the reliable portion of the solar record since AD 1830 appears modulated by a longer period¹⁶, most minimum-to-minimum cycle lengths last century grouping around 11.5 yr and this century near 10–10.5 yr. A further similarity between the Elatina and solar records is the tendency for cycle duration to vary inversely with cycle amplitude. In the Elatina record, stronger ~12-yr VT cycles tend to be of shorter than average duration (Fig. 2d), and sunspot cycles of large amplitude tend to be of short duration, and vice versa¹⁷.

Portions of the plot of ~12-yr VT cycle thicknesses may be compared with the reliable sunspot record since AD 1830. Similarities include the structure of certain Elatina cycle ($26 \times \sim 12$ -yr VT) amplitude maxima and that of the solar maximum culminating in AD 1957, and the sawtooth pattern of cycle amplitudes (which maintains phase across the AD 1957 solar maximum). Such similarities between the Elatina and solar series are evident in Fig. 5. Cross-correlation between the overlapping portions of the curves shown in Fig. 5 yields the highly significant correlation coefficients (Pearson) of 0.95 (curves *a* and *b*) and 0.93 (curves *a* and *c*). That only the reliable portion of the sunspot record may be compared in this way with part of the Elatina curve increases confidence that the Elatina Formation indeed records solar variability.

Overall, the evidence from both the Elatina detailed and long sequences supports earlier conclusions^{1,2} of a direct connection between solar variability, climatic temperature change, and varve-thickness cyclicity during the Marinoan Glaciation in South Australia. In other words, increase in solar activity caused a corresponding increase in climatic temperature that resulted in greater annual meltwater discharge and the deposition of thicker varves. Importantly, recent varves deposited in glacial Skilak Lake in Alaska⁶ reveal a relatively weak solar signal recorded in this way¹⁸. Nonetheless, solar influence on climate must have been much greater during deposition of the Elatina Formation than is evident now. The composition of the late Precambrian atmosphere¹⁹ probably differed greatly from that

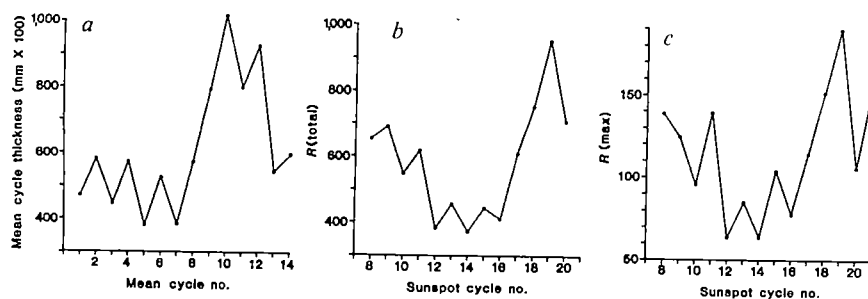


Fig. 5 Comparison of amplitude curves for ~12-yr VT cycles and sunspot cycles. *a*, Mean of five amplitude peaks from the Elatina long sequence (centred around cycles 89, 176, 855, 1,196 and 1,250) across which the phase of the sawtooth pattern is maintained. (A 'mean peak' was determined to reduce random noise in the geological data.) *b*, Total annual mean sunspot number for sunspot cycles since AD 1830. *c*, Annual mean sunspot number *R* at maxima of sunspot cycles since AD 1830.

of today, however, and the global climate may then have been much more sensitive to solar variability than is now observed³.

The Elatina record, in view of its clarity and great length, accordingly may provide invaluable data on which to model the physics of the solar activity cycle^{3,17}. The implications of these new data for solar physics and planetary science will be addressed separately.

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Thermally induced residual topography within oceanic lithosphere

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The lithosphere, following a thermal event, should subside isostatically to its pre-event configuration. However, if the thermal event is sufficient to reset the rigidity of the lithosphere, then the subsidence is forced to follow a different path to the initial uplift; lithospheric rigidity decrease during uplift allows flexural deformation resetting, while during subsidence, an increasing rigidity with time results in flexural interference. This 'thermo-mechanical hysteresis' effect may be responsible for anomalous bathymetry and free-air gravity anomalies associated with hotspot traces, extinct spreading centres and the ocean/continent boundary of rifted (passive continental) margins.

BROAD-SCALE reheating of the lithosphere is the result of thermal processes such as hotspot volcanism, rifting and possibly small-scale mantle convection. Concomitant with thermal modification of the lithosphere is its isostatic uplift and mechanical weakening (rigidity decrease). The actual uplift topography is a function of only the minimum rigidity obtained during heating. Subsequent cooling of the lithosphere allows its subsidence and mechanical recovery (increasing flexural rigidity with time). However, in marked contrast to the uplift, the cumulative subsidence is a function of the entire mechanical recovery history of the lithosphere. Flexural interference between this cumulative uplift and the initial topography produces a residual topography, the amplitude and wavelength of which critically depends on the rate of mechanical recovery and the degree of lithospheric heating.

The production of residual topography, as it involves the entire crust, resembles a buckled elastic plate. Even though such topography will necessarily be associated with large free-air gravity anomalies, the forces responsible for the deformation remain in mechanical and isostatic equilibrium at all times as evidenced by the equal positive and negative components of both the topography and free-air gravity anomaly. Formation of residual topography by the flexural interference of thermally modified lithosphere is here termed 'thermo-mechanical hysteresis' (TMH). It is suggested that TMH may be responsible for the characteristic free-air gravity anomalies observed over extinct spreading centres, for example, within the South China Sea and the Coral Sea basins, the isostatic gravity anomaly observed across the ocean/continent boundary of many passive margins, for example Argentina and the northeastern US margins, and anomalous bathymetric trends within the major

oceanic plates, for example, the linear bathymetric trend associated with the Cocos-Keeling Islands in the Indian Ocean.

Structure of the lithosphere

The interdependence of the thermal structure of the lithosphere and its mechanical properties has been convincingly demonstrated by recent studies of loading within the oceans and continents¹⁻⁵. Furthermore, these studies have established that flexure is the predominant mechanism by which emplaced loads are supported by the lithosphere. The form of the support is dependent on the lithospheric rigidity, which itself increases with lithospheric age. Generally, in the oceans, the plate age defines the thermal structure of the lithosphere through the cooling plate model⁶, while in the continents, rather than absolute plate age being important, it is the time since the last major thermal event as defined by either reset radiometric base-metal ages¹ or episodes of extensive alkalic or basic volcanism⁷.

Observations of the subsidence of seamounts associated with intraplate swells suggest that the swells behave like oceanic crust reset to a younger thermal age^{2,8-11}. The swells, in turn, are related to the broad-scale heating and hence thermal expansion of the lithosphere above hotspots (for example, Hawaii, Snake River province) or hot-lines (for example, Easter Island chain¹², eastern Australia^{13,14}). Thermal rejuvenation results in a lithosphere with properties identical to that of normal lithosphere but with an apparent younger thermal age and correspondingly lower lithospheric rigidity².

Thermal modification of the lithosphere by a general heating event causes an immediate isostatic uplift in an attempt to compensate the thermally expanded lower lithosphere^{14,16}. Isostatic compensation is essentially supplied by a Pratt-type pro-

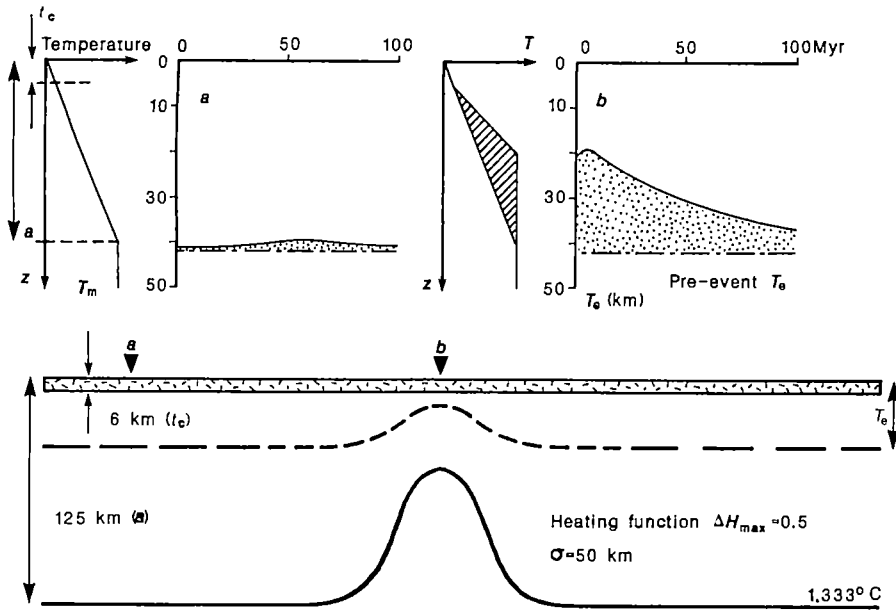


Fig. 1 Schematic representation of the initial rigidity structure of the lithosphere, the distribution of the heating function (parametrized by ΔH_{\max} and σ), the initial thermal structure over the zone of maximum heating and in the distant regions of the plate, and the mechanical recovery of the lithosphere (that is, the variation of T_e , the effective elastic thickness of the lithosphere) with time.

cess in which the uplifted topography compensates the lower densities associated with the thermally modified lithosphere. Even though Pratt isostasy is a local compensation scheme, the form and maximum amplitude of the surface deformation will be determined by the minimum lithospheric rigidity obtained during the thermal resetting process (in both space and time). Because plate rigidity is defined by the depth to a particular isotherm (in this case, the 450 °C isotherm⁵), minimum rigidity resetting occurs some time after the initiation of cooling as heat is removed vertically from the lower lithosphere to the upper lithosphere (Fig. 1). Second, minor heating associated with lateral heat flow eventually modifies rigidities in the distant regions of the plate. Following the maximum deformation stage, cooling of the lithosphere by vertical and lateral heat flow will result in a spatially and temporally varying flexural rigidity. In particular, the temporally increasing flexural rigidity will produce a progressively broader subsidence with time relative to the initial uplift. Flexural interference between this initial uplift and subsequent broader subsidence results in a residual topography. The purpose of this article therefore, is to model quantitatively the thermo-mechanical response of the lithosphere due to thermal loading and to define the production and form of residual topography.

Thermal loading

Several studies have suggested that mid-plate swells are adequately approximated by a gaussian-type heating function¹⁰. For convenience, the same functional form will be assumed for the distribution of the thermal load in this study. Heat input into the lithosphere can be characterized by the dimensionless quantity¹⁶ ΔH , where $\Delta H = 1$ represents complete melting of the lithosphere while $\Delta H = 0$ represents stable thermal equilibrium within the lithosphere. The degree of lithospheric heating can also be expressed in terms of the lithospheric stretching function of McKenzie¹⁵, and, in particular, the two-layer stretching model of Royden and Keen¹⁶, even though no literal stretching is implied. In particular,

$$\Delta H = (1 - 1/\beta) \left(\frac{a - t_c}{a} \right)^2 \exp[-x^2/\sigma^2]$$

$$\Delta H_{\max} = 0.5, \quad \beta_{\max} = 2.5$$

where ΔH_{\max} is the maximal thermal heating, β is typically the stretching of the sub-crustal lithosphere, σ is the half-width of the heating function (with modelling constants defined in Table 1), a is the lithospheric thickness, t_c the oceanic crustal thickness and x is the horizontal coordinate. As the lithosphere/asthenosphere boundary represents an isotherm (T_m), β can define not only the degree of sub-crustal stretching, but also the maximum

vertical migration of this temperature boundary and therefore the degree of subcrustal heating. The use of β does not necessarily imply a tectonic process or setting. However, the actual details of the heating process or the distribution of the thermal anomaly itself are not critical to this argument. Whenever a load, thermal or otherwise, is removed incrementally from the lithosphere while the flexural rigidity is varied, a permanent lithospheric deformation results. This article links the thermal loading (and subsequent unloading as the load is conductively dissipated) of the lithosphere with its thermal and hence mechanical modification (and subsequent recovery). By calculating $\beta(x)$ from ΔH , the initial (or instantaneous) surface uplift is¹⁷,

$$S_i = \frac{\alpha \rho_m T_m}{2(\rho'_m - \rho_w)} \Delta H(x)$$

where α is the thermal expansion coefficient, and ρ_m , ρ'_m and ρ_w are the mantle density at 0 °C, the asthenosphere density and the water density, respectively.

The initial surface uplift S_i , assumes that the lithosphere has zero flexural strength (Pratt or local isostasy) and is termed the

Table 1 Modelling parameters used

Parameter value	Definition
$a = 125$ km	Lithospheric thickness
$t_c = 6$ km	Oceanic crustal thickness
$\kappa = 0.008$ cm ² s ⁻²	Thermal diffusivity
$\alpha = 3.4 \times 10^{-5}$ °C ⁻¹	Thermal expansion coefficient
$T_m = 1,333$ °C	Temperature defining lithosphere
$\tau = 62.8$ Myr	Thermal relaxation coefficient
$Z_e = 450$ °C	Controlling elastic isotherm
$\rho_w = 1.03$ g cm ⁻³	Water density
$\rho_s = 2.5$	Sediment density } ρ_i
$\rho_m = 3.33$	Mantle density at 0 °C
$\rho'_m = \rho_m(1 - \alpha T_m)$	Asthenosphere density
$= 3.179$	
$g = 982$ cm s ⁻²	Acceleration due to gravity
$\gamma = 6.673 \times 10^{-8}$ cm ³ g ⁻² s ⁻²	Gravitational constant
$\Delta H_{\max} = 0.5$	Maximum thermal heating
$\beta_{\max} = 2.5$	Equivalent sub-crustal stretching value
$\sigma = 50$ km	Standard deviation of heating function
$D = ET_c^3/12(1 - \nu^2)$	Lithospheric rigidity
T_e	Effective elastic thickness
$E = 6.5 \times 10^{11}$ dyn cm ⁻²	Relaxed Young's modulus
$\nu = 0.25$	Poisson's ratio

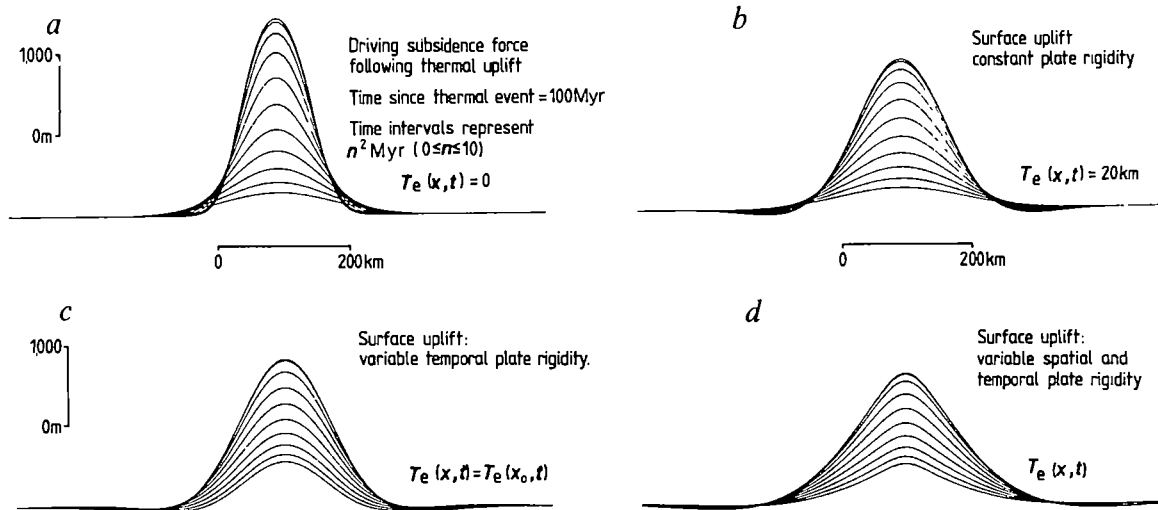


Fig. 2 Following a thermal event, the driving subsidence associated with the cooling and contraction of the lithosphere opposes the initial uplift, itself associated with a thermal buoyancy force. The resulting time-dependent force balance is subsequently modified by the mechanical properties of the lithosphere, producing a time-varying surface topography. The surface topography is illustrated for various assumptions of the mechanical response of the lithosphere at 0, 4, 9, 16, 25, 49, 64, 81 and 100 Myr since the initiation of cooling. Modelling parameters are defined in Table 1. *a*, Surface response of the lithosphere assuming a local isostatic response to loading. As local compensation refers to a zero-rigidity lithosphere, the form of the surface response is equivalent to the resultant force acting on the lithosphere. As lithospheric cooling has been modelled using both vertical and lateral heat flow, the force spreads laterally with time. *b*, Surface response of the lithosphere assuming a constant rigidity. If the thermal event fails to reset the lithospheric rigidity, the uplift and subsidence histories are identical and therefore no residual topography is produced. *c*, Surface response of the lithosphere assuming that its rigidity is reset and subsequently increases with time following the thermal event. The differing uplift and subsidence histories of the lithosphere result in a residual topography, the amplitude and wavelength of which relates to the pre-event rigidity and the degree of resetting. *d*, Surface response of the lithosphere assuming both a spatial and temporal variation in rigidity. The spatial rigidity variations relate to the distribution of the thermal event whereas the temporal variations relate to the thermal properties of the lithosphere. Note that in both *c* and *d* the topographic wavelength decreases with time, implying a progressive decrease in flexural rigidity. Such behaviour is usually interpreted in terms of the flexural response of a (Maxwell) visco-elastic plate to loading. However, in this case, the changing topographic wavelength is simply reflecting the sensitive interrelationship between the mechanical properties of the lithosphere and its temperature structure.

'local uplift'. Although Pratt isostasy (or any local isostatic scheme) generally represents an unrealistic response of the lithosphere to loading, it is very useful in describing the potential uplift of the lithosphere. Convolution of this local uplift with a flexural filter redistributes the surface uplift according to the mechanical properties of the lithosphere. For example, if the lithosphere has constant elastic rigidity D , then the surface uplift is given by (in the frequency domain)¹⁸,

$$S_i^* = \frac{\alpha \rho_m T_m}{2(\rho'_m - \rho_w)} \Delta H(k) \left[1 + \frac{Dk^4}{(\rho_m - \rho_i)g} \right]^{-1}$$

where ρ_i is the density of material infilling the deformation, k is the wavenumber, and g is the acceleration due to gravity. As a consequence of thermally modifying the temperature structure of the lithosphere, D is not constant but varies both spatially and temporally in the plate such that $D = D(x, t)$, t being the time since the initiation of lithospheric cooling.

Cooling of the lithosphere by the vertical and lateral flow of heat away from the thermally perturbed zone results in the creation of an opposing subsidence which progressively negates the initial surface uplift. The local subsidence counteracting S_i is given by¹⁹,

$$S_t(x, t) = \frac{2\rho_m \alpha T_m a}{(\rho'_m - \rho_m)} \sum_{n=1}^{\infty} \frac{2}{n^2 \pi^2} A_n^* (1 - e^{-n^2 t / \tau})$$

where

$$A_n = \frac{1}{n\pi} \{ (1 - \beta) \sin(n\pi z_1) + \beta \sin(n\pi z_2) \}$$

$$z_1 = t_c / a, \quad z_2 = z_1 + (1 - z_1) / \beta$$

$$A_n^* = A_n * X(x, t)$$

$$X(x, t) = \frac{1}{2(\pi \kappa t)^{1/2}} \exp[-x^2 / 4\kappa t]$$

where $X(x, t)$ represents the lateral conduction of heat, A_n the Fourier series of the initial vertical lithospheric temperature

distribution, $*$ the convolution operation, and τ is the thermal relaxation coefficient. As before, the actual subsidence is calculated by convolving $S_t(x, t)$, the local subsidence, with the lateral variations of lithospheric rigidity to give S_t^* . Figure 2a summarizes the spatial and temporal behaviour of the local thermal uplift (maximum value of 2,380 m) within the lithosphere for $\Delta H = 0.5$ ($\beta_{\max} = 2.5$) and $\sigma = 50$ km (and for times n^2 Myr since initiation of cooling, $1 < n < 10$) and clearly shows the effects of both vertical and lateral heat flow. Because the isostatic compensation in this example is assumed to be local, the uplift is proportional to the thermally generated buoyancy force acting on the lithosphere. With infinite time, the residual local uplift ($S_i - S_t(x, t)$), and therefore the buoyancy force, are identically zero everywhere.

Lithospheric subsidence

Referring to Fig. 2, we can monitor the subsidence of the lithosphere following a thermal event for a variety of mechanical conditions. If we insist on the mechanical homogeneity of the lithosphere both laterally and with time, the residual subsidence ($S_i^* - S_t^*(x, t)$) will again be reduced to zero given sufficient time even with flexural redistribution of the surface uplift (Fig. 2b). With $T_e = 20$ km and after 100 Myr, a topographic surface with amplitude 254 m is all that remains from an initial uplift of 1,824 m. If, however, we relax the requirement for temporal homogeneity of the lithospheric rigidity, then the flexural modification of the local subsidence changes continually with time. In this example, the pre-event effective elastic thickness of T_e (42.2 km) is thermally reset to 20 km. Mechanical recovery of the lithosphere over a 100-Myr period increases T_e from 20 to 36.9 km (Fig. 1), producing significant residual topography (431 m, Fig. 2c) relative to Fig. 2b.

Thermal modification of the lithospheric temperature structure will obviously be greatest nearest the thermal perturbation. Necessarily, therefore, the mechanical properties of the lithosphere must be a function not only of time since the initiation of cooling, but also of the distance from the perturbation. The

redistribution of the local subsidence, $S_i(x, t)$, by a heterogeneous elastic plate to give the flexural subsidence, $S_i^*(x, t)$ is given by,

$$\frac{\partial^2}{\partial x^2} \left[\frac{D(x, t)}{(\rho_m - \rho_l)g} \frac{\partial^2}{\partial x^2} S_i^*(x, t) \right] + S_i^*(x, t) = S_i(x, t)$$

The lateral and temporal variations of plate rigidity, $D(x, t)$, are calculated by monitoring the depth to the 450 °C lithospheric isotherm⁵. The above differential equation is most conveniently solved using a finite-difference algorithm²⁰. The resulting topography ($S_i^* - S_i^*(x, t)$) is now the result of a complex interaction between vertical and lateral heat flow and the spatial variations of plate rigidity about the thermal anomaly. Figure 2d represents the full model prediction for thermally reset oceanic lithosphere, producing in this example residual topography of 335 m. While the residual topography is only relatively small (100–200 m), note that this deformation was produced with only a modest degree of sub-crustal heating. Areas associated with high-temperature contrasts, such as during the creation of a passive continental margin or during the death of a mid-oceanic spreading centre, will enhance this hysteresis effect and, while probably never dominating, will always be a complicating factor in loading situations when heat is involved.

Apparent visco-elastic behaviour

A curious feature of the residual topography in Fig. 2c, d is that the wavelength appears to decrease progressively with time implying, therefore, an apparent decrease in flexural rigidity with time even though we are able to monitor its actual increase. That is, the surface deflection is mimicking the temporal response of a visco-elastic (Maxwell) plate. This observation simply underlines the sensitive interrelationship between lithospheric temperature structure and its flexural response and may prove to be useful in rationalizing the apparent contradiction posed by those flexural studies which vehemently refute the elastic model in favour of a visco-elastic (Maxwell) model^{21–26}.

Apparent visco-elastic behaviour of the lithosphere is not limited to TMH. McNutt²⁷, in her analysis of seamount flexure, noted that load rejuvenation occurs as the heat introduced into the lithosphere during seamount emplacement is vertically redistributed. The degree of load rejuvenation is therefore sensitive to the level at which the temperature anomaly is introduced. Similarly, foreland basins, produced as an isostatic response to thrust sheet/nappe emplacement²³, modify the temperature structure of the foreland lithosphere (at a rate determined by the thermal diffusivity of the lithosphere) as the overlying sediment infill and thrust sheets thermally re-equilibrate²⁸. In both cases, lithospheric heating induces a decrease in rigidity with time which mimics the behaviour of a Maxwell visco-elastic plate.

While thermo-mechanical hysteresis is concerned with load removal during cooling of the lithosphere, a similar flexural interference is obtained during (non-thermal) load emplacement. For example, Liu *et al.*²⁹ successfully modelled the negative gravity anomaly over the 85° E ridge by investigating the flexural interference between the basement deformation produced by the formation of the ridge and the regional deformation associated with its subsequent burial by sediments. Thermo-mechanical hysteresis, therefore, is only a special case of the flexural interference of lithospheric deformations associated with temporally and/or laterally varying loads and a temporally varying lithospheric rigidity.

Isostatic equilibrium

The isostatic state of the heated lithosphere will be a complicated interaction between the Pratt effect of the thermally modified subcrustal lithosphere and the regional isostatic effect of the evolving crustal deformation. In the limit, however, only the crustal deformation remains. Figure 3 illustrates the residual topography and associated free-air gravity effect produced by thermal re-equilibration of oceanic lithosphere over a 400-Myr period since cooling and for all practical purposes, represents

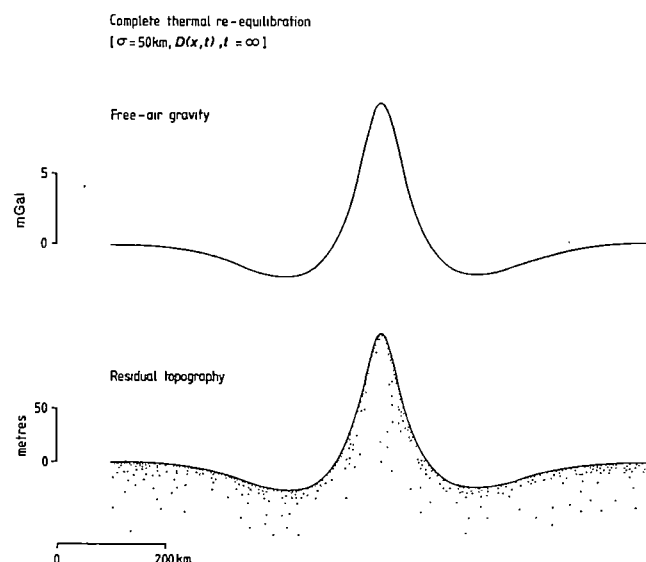


Fig. 3 The resultant free-air gravity effect and residual surface topography following thermal re-equilibration of the lithosphere ($t = 400$ Myr) in which the original thermal perturbation modified both the spatial and temporal flexural properties of the lithosphere. The residual topography and its free-air effect remain an isostatic process in the sense that the topography and free-air gravity laterally integrate to zero (remembering that the Moho parallels the surface).

complete thermal re-equilibrium of the lithosphere. If, during the thermal event, the lithospheric rigidity failed to be reset (that is, remains constant), no residual topography (and hence gravity effect) would be produced. In contrast, any rigidity variation (in space or time) results in a residual topography, in this example being 162 m with a corresponding free-air gravity effect of 20 mGal. Compensation is maintained by the strength of the lithosphere, not in the usual way, but by the production of a surface deformation which laterally integrates to zero (here termed mechanical equilibrium).

Applications

Thermo-mechanical hysteresis results from the thermal re-equilibration of the lithosphere following a heating event. This concept, when applied to the oceanic lithosphere in particular, may help to explain several anomalous bathymetric and gravimetric trends within the Indian Ocean (for example, Cocos-Keeling Islands and associated bathymetric trends), extinct spreading centres (for example, Coral Sea Basin) and ocean/continent boundaries of passive margins (for example, Argentinian margin). No attempt has been made to model the bathymetry or gravity anomalies in detail, but the similarity in characteristics predicted by thermo-mechanical hysteresis, namely, low-amplitude but long-wavelength topography, relatively large-amplitude free-air gravity anomalies relative to the topography (or equivalently, an anomalously high gravitational admittance) and an isostatic compensation reminiscent of mechanical isostasy (a balanced deformation of the entire crust generally reported as uncompensated topography), and the above geological features, strongly suggests that the same process, TMH, may be operative.

Cocos-Keeling hotspot trend

Anomalous north-east-south-west trending bathymetry (1–2 km) within the northern Indian Ocean and generally paralleling the absolute motion of the Indo-Australian plate (Fig. 4a) is associated with the Cocos-Keeling Islands, the trend and islands being produced by a presumed hotspot of Lower Tertiary age. Although the bathymetry of the trend is minor relative to the Ninety-East Ridge, the associated free-air gravity anomaly is excessively large (Fig. 4a). To remove the effect of the Cocos-

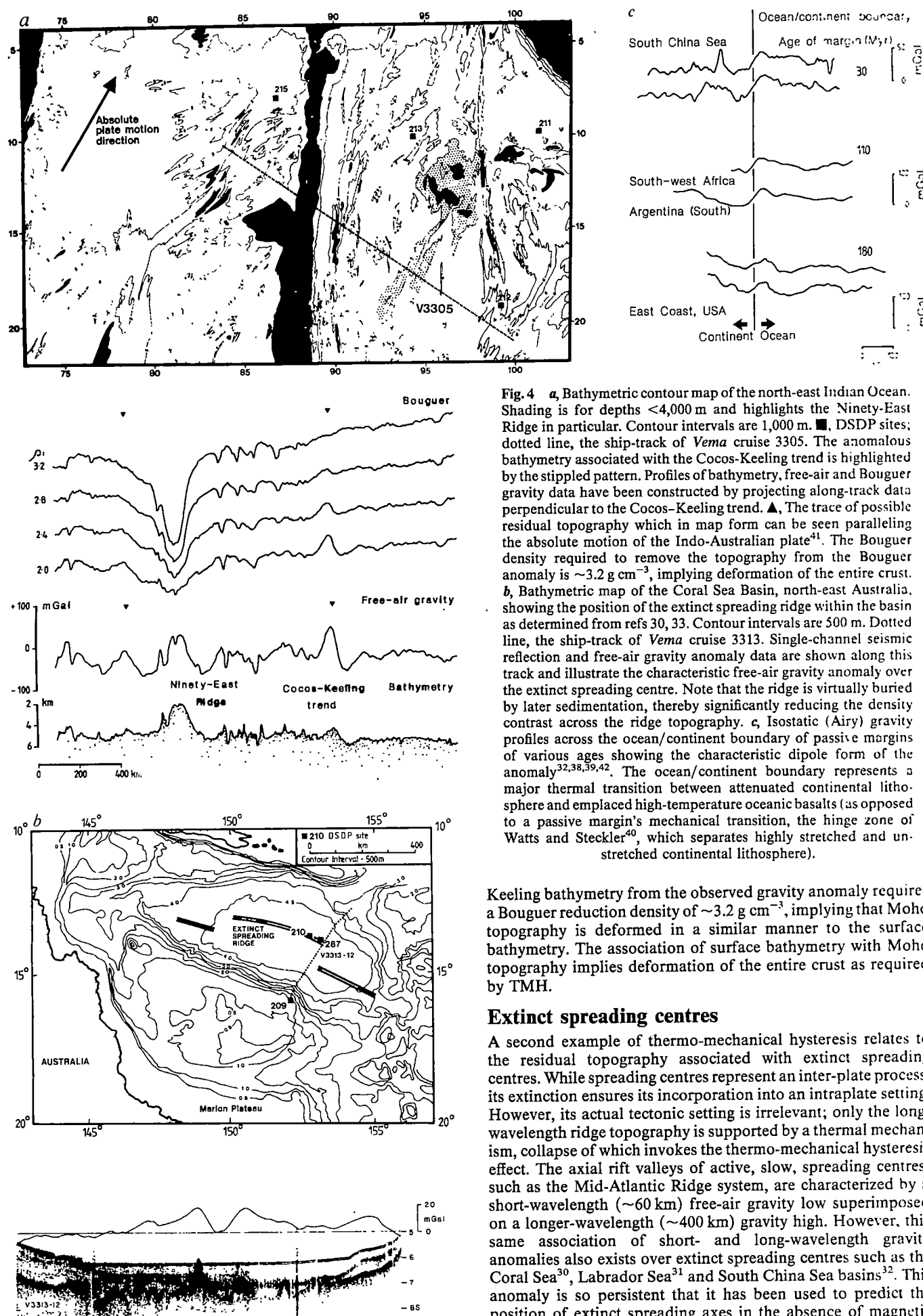


Fig. 4 *a*, Bathymetric contour map of the north-east Indian Ocean. Shading is for depths <4,000 m and highlights the Ninety-East Ridge in particular. Contour intervals are 1,000 m. ■, DSDP sites; dotted line, the ship-track of *Vema* cruise 3305. The anomalous bathymetry associated with the Cocos-Keeling trend is highlighted by the stippled pattern. Profiles of bathymetry, free-air and Bouguer gravity data have been constructed by projecting along-track data perpendicular to the Cocos-Keeling trend. ▲, The trace of possible residual topography which in map form can be seen paralleling the absolute motion of the Indo-Australian plate⁴¹. The Bouguer density required to remove the topography from the Bouguer anomaly is $\sim 3.2 \text{ g cm}^{-3}$, implying deformation of the entire crust. *b*, Bathymetric map of the Coral Sea Basin, north-east Australia, showing the position of the extinct spreading ridge within the basin as determined from refs 30, 33. Contour intervals are 500 m. Dotted line, the ship-track of *Vema* cruise 3313. Single-channel seismic reflection and free-air gravity anomaly data are shown along this track and illustrate the characteristic free-air gravity anomaly over the extinct spreading centre. Note that the ridge is virtually buried by later sedimentation, thereby significantly reducing the density contrast across the ridge topography. *c*, Isostatic (Airy) gravity profiles across the ocean/continent boundary of passive margins of various ages showing the characteristic dipole form of the anomaly^{32,38,39,42}. The ocean/continent boundary represents a major thermal transition between attenuated continental lithosphere and emplaced high-temperature oceanic basalts (as opposed to a passive margin's mechanical transition, the hinge zone of Watts and Steckler⁴⁰, which separates highly stretched and unstretched continental lithosphere).

Keeling bathymetry from the observed gravity anomaly requires a Bouguer reduction density of $\sim 3.2 \text{ g cm}^{-3}$, implying that Moho topography is deformed in a similar manner to the surface bathymetry. The association of surface bathymetry with Moho topography implies deformation of the entire crust as required by TMH.

Extinct spreading centres

A second example of thermo-mechanical hysteresis relates to the residual topography associated with extinct spreading centres. While spreading centres represent an inter-plate process, its extinction ensures its incorporation into an intraplate setting. However, its actual tectonic setting is irrelevant; only the long-wavelength ridge topography is supported by a thermal mechanism, collapse of which invokes the thermo-mechanical hysteresis effect. The axial rift valleys of active, slow, spreading centres, such as the Mid-Atlantic Ridge system, are characterized by a short-wavelength ($\sim 60 \text{ km}$) free-air gravity low superimposed on a longer-wavelength ($\sim 400 \text{ km}$) gravity high. However, this same association of short- and long-wavelength gravity anomalies also exists over extinct spreading centres such as the Coral Sea³⁰, Labrador Sea³¹ and South China Sea basins³². This anomaly is so persistent that it has been used to predict the position of extinct spreading axes in the absence of magnetic

anomalies^{32,33}. Once spreading has ceased within a rift axis, heat is rapidly dissipated, allowing the ridge topography to subside and thereby destroying its characteristic waveform. In general, the isostatic compensation of spreading ridge topography is poorly understood. Interpretation of admittance functions obtained across active spreading systems^{34,35} suggests low lithospheric strengths ($T_e < 10$ km). Seismic studies have shown, however, that oceanic crust is of normal thickness at least to within ~ 10 m of the ridge axis³⁶. As the crustal thickening predicted by isostatic models (in which ridge topography is represented as a surface load) is not observed, such models fail to describe adequately the isostatic state of the ridge. The most promising approach has been to model the ridge topography as the response of a broken elastic plate to a sublithospheric buoyancy force³⁷, analogous to the thermal event of Fig. 2a. The extinction of the spreading ridge system means the eventual dissipation of the thermally generated buoyancy force, ridge subsidence, and as the lithosphere mechanically recovers, the 'freezing-in' of the ridge topography by thermo-mechanical hysteresis.

The extinct spreading centre of the Coral Sea Basin (Fig. 4b) is associated with a short-wavelength (~ 50 km) free-air gravity anomaly low of 15–20 mGal and a longer-wavelength (~ 350 km) gravity high³⁰ (Fig. 4b). Since the rift valley topography has been buried by turbidites infilling the Coral Sea Basin from Australia and New Guinea, the source of the free-air gravity anomaly is approximately shared between the crust-sediment and crust-mantle interfaces. Calculating the gravity effect of each interface using the Bouguer approximation gives:

Interface (1): $42.2 \text{ mGal km}^{-1} \text{ g}^{-1} \text{ cm}^{-3}$

Upward continuation: $\exp(-kd)$; k , the wavenumber, is defined as $2\pi/L$, L being wavelength (~ 50 km) and, therefore, $k = 0.126$, and d , the mean depth of the interface, is ~ 5 km.

$$42.2(2.8 - 2.5)1.5 \exp(-0.126 \times 5) = 10 \text{ mGal}$$

Interface (2): $d = 10$ km, $k = 0.126$

$$42.2(3.33 - 2.8)1.5 \exp(-0.126 \times 10) = 9.5 \text{ mGal}$$

The combined free-air gravity effect is 19.5 mGal, indicating that the short-wavelength acoustic topography of the Coral Sea rift valley is conformable with the Moho topography. Given the accumulating evidence for the existence of an elastic layer supporting both active and inactive rifts³¹, it would appear that the 'frozen' ridge topography is most simply explained (though not explicitly modelled) in terms of the thermo-mechanical hysteresis of this elastic layer and is responsible for the observed crustal configuration and free-air gravity anomaly.

Ocean/continent boundaries

The final example relates to the persistent free-air gravity anomaly associated with ocean/continent boundaries, often highlighted by the calculation of an Airy isostatic anomaly, of many passive continental margins. The ocean/continent bound-

ary is characterized by a steep landward gradient, the midpoint of which identifies the position of the boundary as collaborated by magnetic anomalies and reflection seismics^{32,38,39}. Some margins also have an associated basement high which appears to be uncompensated³⁹. The form of the isostatic anomaly approximates a dipole, centred on the ocean/continent boundary, with the positive and negative components relating to the oceanic and continental sides respectively (Fig. 4c). A cause in terms of differential subsidence and flexure between the oceanic and continental crust is considered unlikely because, as noted by Watts and Steckler⁴⁰, the mechanical properties of thinned continental and oceanic lithosphere are similar and, therefore, it is not obvious why a flexural deformation between unstretched continental crust and thinned continental/oceanic crust should be centred on the ocean/continent boundary. In the case of the South China Sea (margin age ~ 30 Myr), the isostatic anomaly³² has an amplitude of ~ 30 mGal, ~ 400 km wavelength, and landward gradient $0.85 \text{ mGal km}^{-1}$ (Fig. 4c). In contrast, the significantly older Argentinian margin (age ~ 110 Myr) has an anomaly amplitude of ~ 60 mGal, 400 km wavelength, and gradient $0.95 \text{ mGal km}^{-1}$ (that is, the admittance tends to increase with increasing margin age). The steep gradient demands a relatively shallow source (crustal) while the wavelength and dipole nature of the anomaly suggests that compensation is related to the deformation of the crust. The association of this anomaly with a thermal process across the ocean/continent boundary (the initial continental rifting) again suggests that the ocean/continent boundary anomaly is a product of thermo-mechanical hysteresis.

Conclusions

Long after the cessation of inter- or intraplate thermal activity, the surface of the lithosphere will remain distorted with an amplitude and wavelength dependent on the mechanical properties of the lithosphere and the degree of reheating. This residual topography will remain indefinitely unless reset and hence redistributed by a subsequent thermal event. As the production of residual topography is a hysteresis process, the previous deformation is only replaced by a new deformation—the surface can never return to a stress-free state. Suitable thermal events include intraplate hotspots, formation of ridge topography at spreading centres, and continental (oceanic) rifting leading to the formation of an ocean/continent (ocean/ocean) boundary. Given the ability of the lithosphere to recover mechanically from a superimposed thermal event, the existence of TMH is an undeniable consequence. However, further work will be required to determine its exact importance.

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The *c-myc* oncogene driven by immunoglobulin enhancers induces lymphoid malignancy in transgenic mice

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Transgenic mice bearing the cellular myc oncogene coupled to the immunoglobulin μ or κ enhancer frequently develop a fatal lymphoma within a few months of birth. Since the tumours represent both immature and mature B lymphocytes, constitutive c-myc expression appears to be highly leukaemogenic at several stages of B-cell maturation. These myc mice should aid study of lymphoma development, B-cell ontogeny and immunoglobulin regulation.

THE arguments that a small number of cellular genes, the proto-oncogenes, are crucial to the development of neoplasia are persuasive^{1,2}. Nevertheless, the evidence that natural malignancies are caused by somatically altered proto-oncogenes has remained largely circumstantial³. Transgenic mice (reviewed in ref. 4) provide the means to test directly the efficacy of cellular oncogenes *in vivo*. Since a gene injected into a fertilized ovum typically integrates into a host chromosome within a few cell divisions, all tissues of the transgenic mouse usually acquire the gene, but the regulatory elements of the gene may direct tissue-specific expression⁴. Introduced immunoglobulin genes, for example, are expressed only in the lymphoid cells of transgenic mice⁵⁻⁸.

Recently, both a viral and a cellular oncogene have been shown to elicit characteristic tumours in transgenic mice. The type of tumour induced by the large-T antigen gene of simian virus 40 (SV40) depends on the element controlling its expression; with the SV40 enhancers, papillomas of the choroid plexus develop^{9,10}, while the insulin regulatory region leads to pancreatic tumours¹¹, and the metallothionein-I control region yields still other neoplasms¹². The cellular *myc* oncogene linked to the regulatory region within the long terminal repeat (LTR) of the mouse mammary tumour virus promotes mammary carcinomas¹³. These results suggest that tissue-specific regulators can target the action of oncogenes to particular cell types.

We wished to establish whether the *c-myc* gene can be introduced into the mouse genome in a form that promotes lymphoid malignancy. Altered regulation of *c-myc* expression has been strongly implicated in lymphoid neoplasia. In retrovirus-induced avian B lymphomas^{14,15} and some rodent T lymphomas^{16,17}, proviral insertion near *c-myc* brings it under the control of the promoter or enhancer within the retroviral LTR. In most human Burkitt's lymphomas and murine plasmacytomas, the *myc* gene has been activated by translocation to the immunoglobulin heavy (*IgH*)-chain locus (reviewed in refs 18-20). The way in which the *IgH* locus activates *c-myc* expression is poorly understood, but the lymphoid-specific *H* locus enhancer (*E μ*)²¹⁻²⁴ may well be the activating element in those translocations that couple it to *c-myc*.

By constructing transgenic mice bearing different forms of the *c-myc* gene, we have established that subjugation of this proto-oncogene to immunoglobulin enhancers converts it into a potent leukaemogenic agent for B lymphoid cells. Our results also support the notion that expression of the normal *myc* gene is subject to feedback regulation^{18,25} and bear upon the issue of whether an activated *c-myc* gene alone is sufficient to cause tumours. Since the predisposition is heritable, the novel lines of *myc* mice described here should prove valuable in unravelling early events in lymphoma development, in defining early stages

of lymphoid ontogeny, and in clarifying the mechanisms that regulate immunoglobulin gene expression.

Enhancers make *c-myc* tumorigenic

The seven constructs we have introduced into mice include an intact murine *c-myc* gene (top), a truncated form bearing the coding region (exons 2 and 3) and five versions with *c-myc* coupled to other regulatory regions (Fig. 1a). In the *E μ -myc* construct, which is equivalent to the rearranged *c-myc* allele in the plasmacytoma ABPC17 (ref. 26), the *H* locus enhancer lies upstream of exon 1, while in the LTR-*myc* construct the enhancer region of a murine retroviral LTR is positioned similarly¹⁶. In *E κ -SV-myc*, the lymphoid-specific immunoglobulin κ enhancer^{6,27,28} is coupled through the SV40 promoter to *myc* exons 2 and 3. SV-*myc* and MT-*myc* are analogous constructs containing the enhancer plus promoter of SV40 or the murine metallothionein-I gene, respectively. In four of the constructs the *myc* 3'-untranslated region was marked (Fig. 1b) with an irrelevant sequence (0.6 kilobases (kb) of Φ X174 phage DNA) to distinguish its transcripts from those of the endogenous *myc* gene.

To make transgenic *myc* mice, (C57BL \times SJL)F₂ eggs were injected with a construct, implanted in pseudopregnant females, and transgenic pups identified by hybridization to tail DNA²⁹. Many of these primary transgenic animals developed tumours, and the incidence is expressed in Fig. 1a as a fraction of the mice bearing each construct.

Significantly, both the μ and κ enhancer constructs elicited tumours and all involved lymphoid tissue. The *E μ -myc* gene was remarkably potent, 13 of 15 primary transgenic animals developing lymphomas (see below). These mice died, or became terminally ill (and hence were killed) between 6 and 15 weeks of age (median 11 weeks). With *E κ -SV-myc*, 6 of 17 transgenic animals died with lymphomas between 11 and 44 weeks of age (median 23 weeks). The efficacy and specificity of these constructs are almost certainly due to the immunoglobulin enhancers, because no tumours have arisen during 10 months of observation in the mice carrying either the tagged normal *myc* gene or the truncated form typical of most murine plasmacytomas^{19,20}. Since both immunoglobulin constructs were effective in multiple primary transgenic animals, each representing an independent insertion event, these enhancers must function in diverse chromosomal environments, as can intact μ and κ genes⁵⁻⁸.

The non-immunoglobulin constructs gave few tumours. The LTR enhancer-*myc* construct, which is derived from a T lymphoma¹⁶, gave rise to only one tumour, a thymic lymphoma. With the SV-*myc* construct, which is equivalent to one that transforms very effectively *in vitro*³⁰, 3 of 21 mice developed tumours: a lymphosarcoma, a renal carcinoma and a fibrosar-

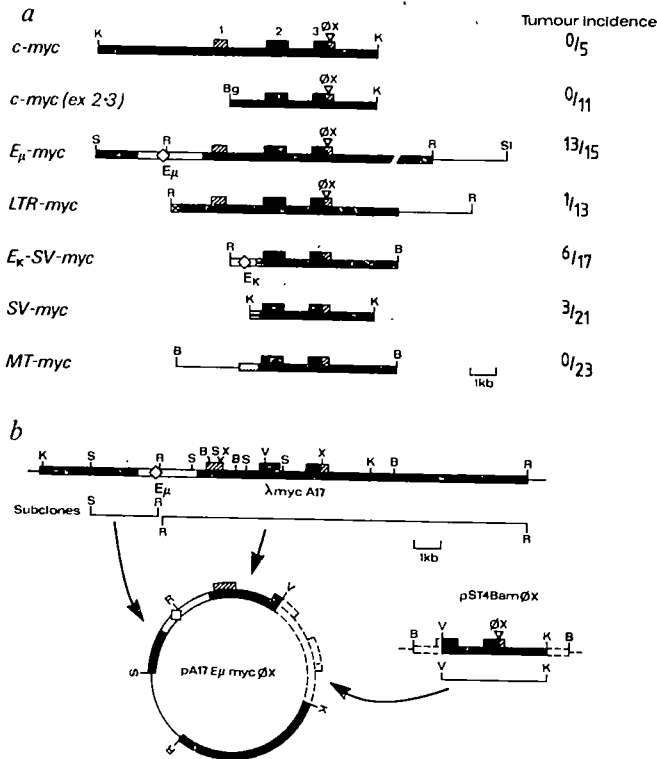


Fig. 1 **a**, The *c-myc* constructs injected into fertilized ova and tumour incidence in resulting transgenic mice. The solid bar denotes *c-myc* sequences with exons raised, untranslated regions striped, the ΦX174 marker DNA (see **b**) as a triangle, and vector sequences as a thin line. The linear fragments shown were isolated from plasmid constructs (described below) before injection. The *E_μ-myc* construct contains 2.3 kb of immunoglobulin DNA (open bar) spanning the H-chain enhancer (diamond), inserted 361 base pairs (bp) 5' to *c-myc* exon 1, as in plasmacytoma ABPC17 (see **b** and ref. 26) and extends 7.2 kb 3' to *c-myc*. The *LTR-myc* construct has the enhancer-containing 210 bp of the Soule murine leukaemia virus LTR (cross-hatched) inserted ~1.3 kb 5' to *c-myc*, as in T lymphoma ST4 (ref. 16). The bottom three constructs involve fusion to the *Xba*I site directly 5' to *myc* exon 2 (ref. 45). *E_κ-SV-myc* has an ~900-bp fragment of *κ* locus DNA (open bar) containing the *κ* enhancer (diamond) linked to the SV40 promoter (181-bp *Sph*I/*Stu*I fragment); *SV-myc* has the complete SV40 promoter/enhancer region (347-bp *Kpn*I/*Stu*I fragment); and *MT-myc* bears metallothionein-I sequences (stippled), from -750 to +6 (ref. 52), including its heavy metal-regulated promoter. **b**, Construction of *c-myc* plasmids. To mark the ABPC17 *c-myc* gene (ref. 26) within its 3'-untranslated region, the *Eco*RI fragment spanning *c-myc* was subcloned, and its *Eco*RV/*Kpn*I fragment exchanged for one bearing the ΦX174 marker. The latter was taken from pST4BamΦX, constructed by ligating a ΦX174 *Hae*III/*Xho*I fragment (nucleotides 4,984-162) via *Xho*I linkers to a *Xho*I-restricted *Bam*HI subclone isolated from the T lymphoma ST4 (ref. 16). Finally, the ΦX-marked *Eco*RI fragment was inserted at the *Eco*RI site of the ABPC17 *Sac*I/*Eco*RI subclone to give pA17E_μmycΦX. For injection, this plasmid was linearized with *Sal*II, which cuts within the pUC12 vector. The *c-myc* (ex 2+3) fragment was excised from pA17E_μmycΦX with *Bgl*III and *Kpn*I. The marked normal *c-myc* construct (**a**, top) was prepared analogously by replacing the 5' segment of pST4BamΦX (up to the unique *Eco*RV site in exon 2) with an 11-kb *Eco*RI/*Eco*RV fragment spanning the 5' end of the unrearranged *c-myc* gene; the 11.2-kb *Kpn*I fragment was then excised for injection. The *LTR-myc* plasmid was constructed by inserting the ΦX marker into an ~14-kb rearranged *c-myc* fragment from ST4 (ref. 16) by *Eco*RV/*Kpn*I exchange with pST4BamΦX as above, and the isolated *Eco*RI fragment injected. The *E_κ-SV-myc* plasmid was constructed by replacing the SV40 enhancer (*Sph*I/*Kpn*I) with a 900-bp fragment (nucleotides 3,390-4,290 in ref. 53) containing the *κ* enhancer. For *MT-myc*, the *Xba*I/*Bam*HI fragment spanning *myc* exons 2 and 3 was inserted into a MT vector containing a *Xba*I linker at +6 and a 3' *Bam*HI site; it was linearized at *Bam*HI for injection. Restriction endonuclease sites are: K, *Kpn*I; Bg, *Bgl*III; S, *Sac*I; R, *Eco*RI; Sl, *Sal*II; B, *Bam*HI; X, *Xho*I; V, *Eco*RV.

coma. This diversity echoes the wide range of cells in which the SV40 enhancer functions. The absence of tumours of the choroid plexus with this construct may mean that the *myc* gene product is less effective than the large-T antigen^{9,10} in transforming brain epithelial cells. We were surprised that no tumours ensued with the MT-*myc* construct, because that control region has induced expression of other genes in transgenic mice^{12,31,32}, but the vector sequences present in this construct (Fig. 1a) may have had a suppressive effect³³.

Aggressive lymphoma

The striking efficacy of the *E_μ-myc* construct led us to concentrate on mice bearing it. To establish whether the predisposition to lymphoma development was heritable, we analysed progeny from primary *E_μ-myc* mice that survived long enough to allow breeding. In eight independent lineages, lymphomas have developed with high fidelity. Indeed, in three generations of descendants, 59 of 63 mice (94%) have died by 4 months of age. The pathology is very similar in all lineages.

The hallmark of the disease in *E_μ-myc* mice is massive enlargement of the lymph nodes. The onset, most readily assessed by palpation of the inguinal lymph nodes, usually occurs between 3 and 18 weeks of age. A few weeks later, most mice develop 'water wings', prominent lumps on both shoulders and the neck (Fig. 2a), reflecting swelling of the brachial and cervical lymph nodes, and most other nodes are grossly enlarged (Fig. 2b). The spleen usually shows modest (about twofold) expansion and the thymus is markedly enlarged in some mice. The enlarged lymphoid organs are replete with lymphoblasts and the blood contains large numbers of similar cells. Variable invasion of other tissues is often observed. The typical pathological diagnosis is therefore multicentric lymphosarcoma (disseminated lymphoma) with associated leukaemia. Occasionally, mice have developed a thymoma without notable involvement of peripheral lymphoid organs and some have succumbed to a bowel obstruction caused by intestinal involution (intussusception) over focal growths of lymphoblasts in the wall. The typical pathology is somewhat like that induced by Abelson murine leukaemia virus³⁴.

Transplantation into syngeneic mice established that the enlarged lymphoid organs of *E_μ-myc* mice represented frank tumours rather than massive proliferations of 'normal' cells. Rapidly growing tumours arose in the (C57×SJL)F₁ hybrid recipients a few weeks after injection of 10⁵-10⁶ cells from each of 10 *E_μ-myc* mice, including all those in Table 1. Indeed, 10² cells from one tumour tested at lower cell numbers (4Met in Table 1) initiated tumours within 2-3 weeks. The disease in *E_μ-myc* mice is patently highly malignant.

Tumours from single B-lymphoid clones

We wished to identify the cell lineage(s) from which the tumours were derived. The pathology was highly suggestive of a lymphoid disease but did not rule out myeloid involvement. Transcriptional studies of the *μ* constant-region (*C_μ*) locus³⁵⁻³⁷ and gene transfer studies^{7,24} suggest that *E_μ* can operate in many T lymphocytes and certain other haematopoietic cells as well as B cells, but all the tumours so far analysed in detail are B lymphoid in origin. All cell lines derived from them are non-adherent and have an absolute requirement for 2-mercaptoethanol, a strong indication of lymphoid rather than myeloid origin. As expected for B cells, all tumours and cell lines displayed rearrangement and expression of the *IgH* and/or *κ* loci, as illustrated in Figs 3 and 4 and summarized in Table 1 for the tumours of five mice. Arguing against a T-cell origin, no tumour so far studied has displayed the Thy-1 surface antigen, nor exhibited rearrangement or transcription of the T-cell antigen receptor *β* locus (data not shown). Moreover, since the *κ* locus (unlike the *H* locus) appears to be transcriptionally silent in T lymphocytes (refs 6, 38 and our unpublished results), the presence of *κ*-locus transcripts in all the *E_μ-myc* tumour cell lines (see below) strongly favours a B-lymphoid origin.

Table 1 Representative tumours arising in E_{μ} -myc transgenic mice

Pathology	Cell line	Ig genes [‡]		Ig RNA [‡]		sIg #		Cell type
		H	κ	H	κ	1°	2°	
Mouse 1 (285-5-6)♂ Multicentric lymphoma/leukaemia with very large pancreatic lymph node	1 Pan*	R1, R2	G	C_{μ} ++	κ^0+	0	0	Pre-B clone A
	1 Bra*	R3	G	C_{μ} ++	κ^0+	0	0	Pre-B clone B
Mouse 2 (285-5-12)♀ Multicentric lymphoma/leukaemia with thymoma	2 Thy†	R1, R2	G	C_{μ} ++	κ^0+	0	0	Pre-B clone A
	2 Bra*	R3, G§	G	C_{μ} ++	κ^0+	ND	0	Pre-B clone B
	2AxL	R3, G§	G	C_{μ} ++	κ^0+	0	0	Pre-B clone B
	2 Mes*	R3, G§	ND	C_{μ} ++	κ^0+	ND	<5	Pre-B clone B
Mouse 3 (285-5-9)♀ Multicentric lymphoma/leukaemia with intussusception of intestine	3 Mes	R1, R2	R1	μ ++	κ ++	5-10	>90	Pre-B → B clone A
	3 Bra	R1, R2	G	μ ++	κ^0+	5-10	10**	Pre-B → B clone A
Mouse 4 (292-5-5)♂ Multicentric lymphoma/leukaemia with enlarged thymus	4 Thy†	R1, R2	R1, R2	(μ , C_{μ})+++	κ +++	80-90	>90	B clone A
	4 Mes†	R3, R4	R3, R4	(μ , C_{μ})+++	κ +++	80-90	>90	B clone B
	4 Bra†	R3, R4	ND	(μ , C_{μ})+++	κ +++	80-90	>90	B clone B
	4 Met†	R3, R4	ND	(μ , C_{μ})+++	κ +++	80-90	>90	B clone B
Mouse 7 (292-1-1-11)♂ Multicentric lymphoma/leukaemia with thymoma and lymphoblast infiltration of perinodal fat	7 Mes*	R1, R2	R	C_{μ} ++	κ ++	0	0	Pre-B clone A
	7 BM	R1, R2	R	C_{μ} ++	κ ++	ND	ND	Pre-B clone A
	7 ThyD*	R1, R2	R	C_{μ} ++	κ ++	0	ND	Pre-B clone A
	7 ThyV*	R1, R2	R	C_{μ} ++	ND	ND	ND	Pre-B clone A
	7 Spl*	R1, R2	R	C_{μ} ++	ND	ND	ND	Pre-B clone A

Mice specified here were first- or second-generation descendants of three primary transgenic mice (285-5, 292-5 and 292-1) bred with normal (C57BL × SJL)_{F1} hybrids. Sick mice killed by exsanguination under anaesthesia were dissected for assessment of gross pathology. Tissue sections were stained with haematoxylin and eosin and blood smears stained with Giemsa for histopathological assessment. Cell lines are designated by their origin as tumours in the pancreatic (Pan), brachial (Bra), axillary (AxL), mesenteric (Mes) lymph nodes; diffuse mediastinal tissue (Met), thymus (Thy), spleen (Spl) or bone marrow (BM). ThyD and ThyV derived from discrete masses situated dorsally and ventrally in the position of normal thymus. ND, not determined.

* Primary tumour and cell line shown to have equivalent J_H rearrangements.

† Primary tumour oligoclonal but dominated by the single clone present in cell line.

‡ Rearrangement of J_H alleles was determined by Southern blot analysis of *Eco*RI digests using ³²P-labelled probe A (Fig. 3), while J_{κ} analysis used *Bam*HI digests and probe C (Fig. 4, top). G indicates a fragment the same size as that in germline (liver) DNA from C57BL or SJL mice, while R1-R4 indicate rearranged fragments of different sizes.

§ Other rearranged J_H fragments were present in minor yield. We are investigating whether the fragment in germline position is truly unrearranged.

|| Four rearranged bands were detected and their relationship to each other is under investigation.

μ and κ denote authentic μ and κ mRNA, while C_{μ} denotes all other C_{μ} -bearing RNA species (see refs 35, 37, 39) and κ^0 denotes transcripts of the unrearranged κ locus (see Fig. 4). +++ indicates a level comparable to that in a conventional B lymphoma (WEHI-231), and ++ and + about one-third and one-tenth that level.

% Cells with surface immunoglobulin (sIg) in either the primary tumour (1°) or the cell line derived from it (2°).

** Growth used for RNA and DNA analysis was 10% sIg⁺, but the line subsequently progressed to >90% sIg⁺.

Although E_{μ} -driven *myc* expression might be expected to trigger proliferation of the entire B-cell compartment, the simplicity of the *IgH* locus rearrangement patterns (Fig. 3) provided strong evidence that most tumours are monoclonal. Most contain two fragments bearing a heavy-chain joining region (J_H) in equimolar yield but lack the 6.5-kb 'germline' fragment found in liver (G), precisely as expected for a clonal lymphoid cell line that has undergone diversity-joining region (DJ) or variable-diversity-joining region (VDJ) recombination at both *IgH* alleles. The apparent single rearranged fragment in tumours such as 1BraC probably reflects two co-migrating fragments. While a few primary tumours (for example, 2AxL, Table 1) contained four to six rearranged J_H fragments, indicative of oligoclonality, one or two bands always strongly predominated.

The J_H rearrangement pattern also confirmed that the cultured line (C) represented either the sole or very dominant clone within the primary tumour (T) mass (compare C and T for several tumours in Fig. 3).

The tumour masses at different sites in a *myc* mouse are frequently derived from the same clone. In mouse 7, for example, tumours from five different sites displayed identical J_H rearrangements (Fig. 3, Table 1) and even the liver had been invaded by this clone (Fig. 3). Some mice, however, appeared to bear more than one independent tumour (Table 1). For example, the pancreatic and brachial lymph node tumours in mouse 1 (1Pan and 1Bra) had different J_H rearrangements, while the thymic tumours in mice 2 and 4 differed from the lymph node tumours (Fig. 3). These differences may reflect independent transformation events, or maturation of a single primary clone by further rearrangement, such as recombination of V with DJ segments.

B-lymphocyte transformation

The pre-B cell, the earliest defined stage in B-cell ontogeny, displays rearrangement at the heavy-chain locus (*DJ* or *VDJ*) but lacks surface immunoglobulin (sIg), usually because the κ or λ light chain loci are unrearranged. Fusion of a V_{κ} gene with J_{κ} (or, more rarely, V_{λ} with J_{λ}) permits κ or λ expression and progression to the mature (sIg⁺) B cell. Although *c-myc* has been implicated only in tumours of mature B cells and plasma cells, the E_{μ} -*myc* tumours include some with pre-B characteristics, others that represent mature B cells and one that progressed from the pre-B to the B-cell stage (Table 1). Hence, *myc*-induced tumorigenesis is not stage-specific.

Tumours of pre-B type developed in mice 1, 2 and 7 (Table 1). No sIg was present, and μ_m messenger RNA, which encodes the membrane form of μ -chain, was not detected by blot



Fig. 2 a, Fourth-generation E_{μ} -*myc* mouse, 11 weeks old, with swellings due to grossly enlarged lymph nodes. b, Interior of same mouse, displaying gross bilateral enlargement of (from the top) cervical, axillary, brachial, mesenteric and inguinal lymph nodes.

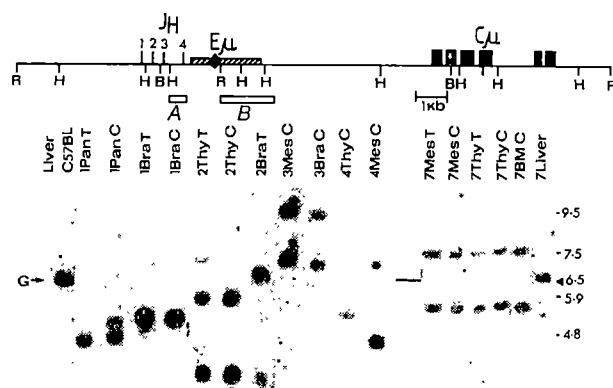


Fig. 3 Rearrangement at the immunoglobulin heavy-chain locus in tumours arising in E_{μ} -myc mice. The diagram indicates the structure of the J_H - C_{μ} locus before rearrangement (germline configuration), with exons indicated as solid boxes, and the enhancer (E_{μ}) as a diamond. Hatched area is the region inserted 5' to c -myc in the E_{μ} -myc construct, and Eco RI (R), Bam HI (B) and $Hind$ III sites are shown. The Southern blot shows J_H rearrangements revealed in Eco RI-cut DNA by hybridization with 32 P-labelled probe A, a 450-bp $Hind$ III/ Nae I fragment, subcloned to eliminate sequences within the E_{μ} -myc insert. An additional fragment detected by probe A in 4Thy (and weakly in 4Mes) is too small (2.8 kb) to be shown by this autoradiograph. An arrow marks the position of the unrearranged J_H -bearing fragment (G) and sizes of marker fragments are given in kilobase pairs (kb). Probe B, a 1.5-kb Eco RI/ Hha I fragment, is the S_{μ} probe used in Fig. 5.

hybridization with a C_{μ} probe (Table 1). As will be documented elsewhere, the C_{μ} -bearing RNAs in these lines are those expected³⁵⁻⁴¹ from either partially rearranged (DJ_H) or unrearranged heavy-chain locus alleles. The tumour in mouse 7 is of an unusual pre-B type, having a κ rearrangement and κ mRNA without μ_m mRNA (Table 1).

Although the cell lines from mice 1 and 2 lack κ rearrangement (Table 1), we found not only the known 8-kb transcript of the germline C_{κ} locus⁴², which initiates upstream of J_{κ} (Fig. 4, top), and a 0.8-kb species⁴³, but also an ~1.1-kb species, the size of κ mRNA (Fig. 4a). Unlike κ mRNA, however, this species bears sequences upstream of J_{κ} , since it hybridizes with probe A + B (Fig. 4b) and probe A alone (not shown). We therefore suggest that processing of the 8-kb RNA generates this previously unrecognized⁴² κ RNA species (Fig. 4, top).

An intriguing tumour that progressed from a pre-B to a B cell developed in mouse 3. The J_H rearrangement patterns assign its mesenteric and brachial lymph node tumours (3Mes and 3Bra) to the same clone (Fig. 3), but only 3Mes had a J_{κ} rearrangement (Table 1). However, after only a few weeks in culture, over 90% of 3Mes cells became sIg⁺, as later occurred for 3Bra. We conclude that this leukaemic pre-B clone generates mature B-cell progeny, as can a few lymphomas induced by Abelson murine leukaemia virus⁴⁴. Thus a c -myc-induced tumour need not be 'frozen' at one stage of differentiation.

Expression of myc gene

Abundant c -myc mRNA was detected in all the E_{μ} -myc cell lines examined, as illustrated for six lines in Fig. 5a. The levels in all the lines, which included tumours from three separate E_{μ} -myc lineages, were comparable and fell within the upper range of that we have observed in plasmacytomas^{45,46}. Due to the 0.6-kb Φ X174 insert within the myc 3' untranslated region (Fig. 1), all the lines contained an ~3-kb myc mRNA, which also hybridized to the appropriate Φ X174 probe (not shown), whereas a normal, unrearranged myc gene yields ~2.3-kb transcripts, as shown for plasmacytoma ABPC103 and B lymphoma WEHI-231 in Fig. 5a. Significantly, no normal c -myc mRNA was detectable in any E_{μ} -myc cell line, even after lengthy autoradiographic exposures (for example, Fig. 5b). Moreover, treatment of four of the lines with the B-cell mitogen lipopolysaccharide (LPS) at 20 μ g ml⁻¹ for 24 or 48 h did not induce normal

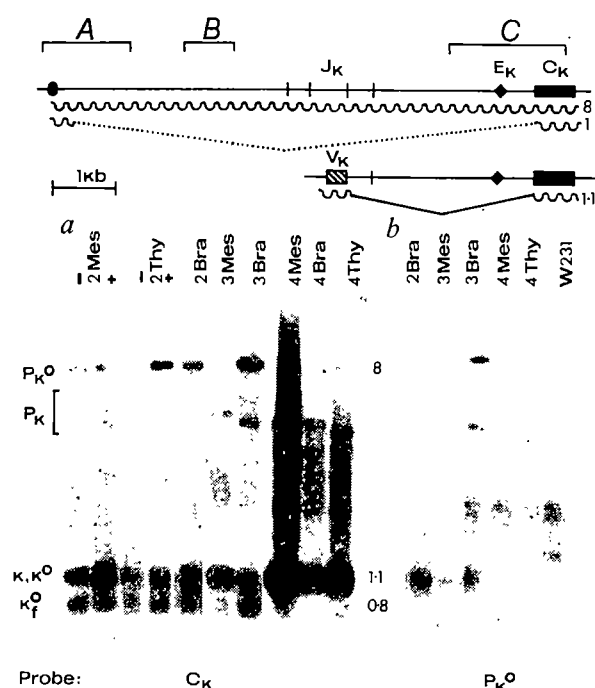


Fig. 4 Transcripts of the κ locus in lymphoma cell lines from E_{μ} -myc mice. The diagram shows that transcripts from the germline C_{κ} locus (top) include a previously described⁴² 8-kb unprocessed (nuclear) RNA initiated at a promoter (filled circle) 3.2 kb upstream from J_{κ} and a proposed spliced ~1.1-kb product (κ) derived from it (see text), while an assembled $V_{\kappa}J_{\kappa}C_{\kappa}$ gene yields 1.1-kb κ mRNA. *a*, C_{κ} -bearing transcripts in the tumour lines. + and - denote cultures grown for 24-48 h in the presence (+) or absence (-) of 20 μ g ml⁻¹ bacterial LPS. The C_{κ} probe was probe C (top), a subcloned 1.5-kb $Hind$ III/ Bgl II fragment. κ_i^0 denotes a 0.8-kb C_{κ} -bearing transcript proposed to be derived from a germline allele (see text), while P_{κ}^0 denotes the unprocessed precursor of authentic κ mRNA. *b*, Transcripts revealed with upstream probes (a mixture of probes A + B above), showing that an ~1.1-kb poly(A)⁺ RNA bears upstream sequences. Probe A alone also labelled the 1.1- and 8-kb species. Probe A is a 1.4-kb $Hind$ III/ Xba I fragment and probe B a 0.7-kb $Hind$ III fragment (see Fig. 1 in ref. 42), prepared from a 12.7-kb Bam HI clone kindly provided by M. Weigert. These probes are free of C_{κ} sequences because they did not reveal a 1.1-kb species in WEHI-231 (W231), which contains abundant κ mRNA, nor in several plasmacytoma lines in which both alleles are rearranged (not shown).

Methods. Tumour lines were established in Dulbecco's modified Eagle's medium supplemented with 50 μ M 2-mercaptoethanol, 100 μ M asparagine and 10% fetal calf serum. Within a few weeks of initiation into culture, the cells were grown in roller bottles and collected while in exponential growth phase ($1-2 \times 10^6$ cells ml⁻¹). Poly(A)⁺ total cellular RNA was isolated from ~10⁹ cells by a method⁵⁴ involving digestion with proteinase K and oligo-dT cellulose chromatography. RNA (2 μ g) heated for 5 min at 65 °C in buffered 2 M formaldehyde/50% formamide was fractionated electrophoretically on a 1.2% agarose/2 M formaldehyde gel and blotted on to nitrocellulose⁵⁵. Hybridization was carried out overnight in 50% formamide/5 \times SSC (SSC is 0.15 M NaCl, 15 mM sodium citrate, pH 7.0) at 42 °C, and washing in 2 \times SSC at 65 °C.

c -myc transcripts (or alter E_{μ} -myc expression), although shorter times might be needed to reveal the c -myc stimulation observed in normal B cells⁴⁷. At least three of the cell lines were clearly responsive to LPS, since their growth rate increased and the levels of some immunoglobulin RNAs increased slightly (Fig. 4 and our unpublished results). The absence of normal c -myc transcripts favours a feedback control model for myc expression (see below).

Two classes of myc RNA of similar size are apparently made from the E_{μ} -myc insert (Fig. 5, top). One class bears myc exon 1 sequences (Fig. 5c) and appears to initiate at the normal myc promoters P_1 and P_2 , judging by S_1 nuclease protection results (W.A., unpublished results). The second class, apparently of

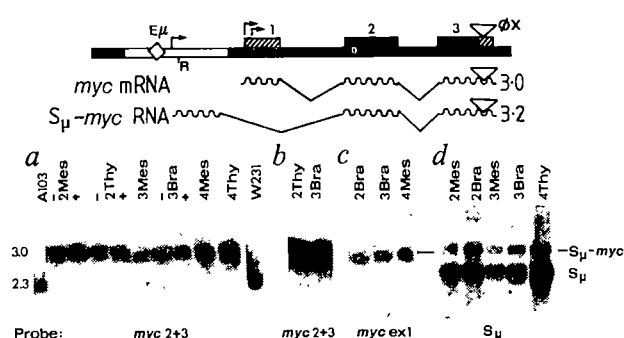


Fig. 5 Transcripts of the *myc* gene in lymphoma cell lines from E_{μ} -*myc* mice. Proposed transcripts from the E_{μ} -*myc* insert are shown at the top (see text). *a*, *myc* transcripts in transgenic lines showing absence of the normal ~2.3-kb *myc* mRNA present in ABPC103 (A103) and WEHI-231, whether the cells were treated with LPS (+) or untreated (-). *b*, Longer exposure of two lanes from *a* (3 days rather than 8 h). *c*, Transcripts bearing exon 1 sequences. *d*, Transcripts bearing S_{μ} sequences, indicating the proposed S_{μ} -(*myc* exons 2+3)-hybrid RNA (S_{μ} -*myc*). The *myc* 2+3 probe was a 1.7-kb *Xho*I fragment from a murine *myc* complementary DNA clone kindly provided by K. Marcu; *myc* ex 1, a *Bam*HI/*Xho*I fragment from exon 1 (see map in ref. 45); S_{μ} , a unique-sequence 1.5-kb *Eco*RI/*Hha*I fragment indicated as bar *B* in Fig. 3 (top).

comparable abundance, may have a 5' segment equivalent to that on certain ' S_{μ} ' RNAs, which bear sequences extending ~0.7 kb downstream from E_{μ} , spliced to C_{μ} (ref. 39). A μ switch region (S_{μ})-*myc* hybrid transcript would explain the novel ~3.0-kb RNA species that hybridizes in all the tumour lines to an S_{μ} probe (Fig. 5*d*) but not to a C_{μ} probe (data not shown). Splicing of the ~0.7-kb S_{μ} sequence to *myc* exon 2 (where the first known splice acceptor occurs), would yield a *myc* RNA marginally larger than the species bearing the normal 0.55-kb *myc* exon 1 (3.2 kb compared with 3.0 kb) and indeed the S_{μ} -bearing species is slightly larger than that detected by a *myc* exon 1 probe on the same filter. The S_{μ} -*myc* RNA is unlikely to encode an altered *myc* polypeptide, because all the ATG sequences within the relevant segment of S_{μ} are followed shortly by stop codons.

Discussion

The novel lymphoid neoplasia described here demonstrates dramatically that subgenation of *c-myc* expression to the immunoglobulin μ or κ enhancer converts this proto-oncogene into a potent leukaemogenic agent *in vivo*. Presumably the malignancy is a consequence of high constitutive *myc* expression within the lymphoid compartment, forced by these enhancers, which are activated by lymphoid factors^{21-23,27,28}. In primary transgenic animals, the E_{μ} -*myc* and E_{κ} -SV-*myc* constructs induced an equivalent pathology, but the E_{μ} -*myc* gene gave a higher tumour incidence and somewhat shorter latent period. This might reflect the greater enhancer activity of E_{μ} over E_{κ} with certain promoters²⁸, or a larger pool of susceptible cells, since E_{μ} is probably activated earlier in lymphoid ontogeny than E_{κ} .

The predisposition to neoplasia in the transgenic lines of E_{μ} -*myc* mice is nearly absolute: over four generations, more than 100 mice involving eight lineages have succumbed within a few months of birth. The typical disease is an aggressive multifocal lymphoma/leukaemia involving lymphoid organs, including the bone marrow, and often invading other tissues. To date, all tumours studied in detail are of the B-lymphoid lineage, but the indications that the E_{μ} enhancer is also active in other haematopoietic cells^{7,35,36,38} leave open the possibility that malignancies of T lymphocytes and myeloid cells arise at a lower frequency. The induction of tumours representing pre-B as well as B cells indicates that *myc*-promoted tumorigenicity is not restricted to one stage.

Our observation that the normal endogenous *myc* genes of the E_{μ} -*myc* tumour lines are silent (Fig. 5) is highly reminiscent

of findings regarding *myc* translocations¹⁸⁻²⁰: in almost all murine plasmacytomas and Burkitt's lymphomas, only the translocated *myc* allele is active^{45,46,48}. One model to account for such results proposes that the normal *myc* gene is subject to negative feedback control by an excess of the *myc* polypeptide or products induced by it^{18,25}. Translocation to the *IgH* locus, or the influence of the E_{μ} enhancer, frees that *c-myc* gene from this restraint, and the ensuing constitutive *myc* expression silences the unaltered allele(s). An alternative model suggests that *c-myc* would not be expressed in a normal cell at the stage of B-cell maturation equivalent to the tumour^{19,49}. A corollary of this model is that *c-myc* expression should be tumorigenic only at those stages where it is normally silent. Judging from Burkitt's lymphomas and murine plasmacytomas, the susceptible stage(s) would be relatively late in B-cell ontogeny, either sIg^{+} B cells or plasma cells. Our evidence that *c-myc* can participate in the transformation of pre-B cells as well as mature B cells argues against this model but is consistent with feedback control.

It has been possible to argue that the major effect of most *myc* translocations is to dissociate the coding region from a negative regulatory element within *myc* exon 1 or its 5'-flanking region, the immunoglobulin locus being merely a passive bystander caught up because of its propensity for rearrangement (see refs 18-20). We found, however, that a *myc* construct lacking the entire 5' portion but bearing all the cryptic promoters within intron 1 (*myc* ex 2+3 in Fig. 1) was completely ineffectual *in vivo*, as was the intact *myc* gene, in marked contrast to the E_{μ} and E_{κ} constructs. These results bolster the case that the immunoglobulin loci have a positive regulatory input. The puzzle that only a minority of *myc* translocations leave *myc* and E_{μ} on the same chromosome may be partially resolved by evidence that *IgH* genes remain active *in vivo* even when deletions remove E_{μ} (ref. 50). If E_{μ} is needed to initiate but not maintain an active chromatin configuration within the C_H locus, the active C_H locus may suffice for *myc* activation.

A critical issue in oncology is whether a single oncogene can shift a normal cell to the fully malignant state. The potency *in vivo* of the acute retroviruses, such as those bearing *v-myc*, seems to argue that one oncogene suffices, but *in vitro* studies suggest that transformation of normal cells usually requires particular combinations of active oncogenes, such as a *myc* plus a *ras* gene³⁰. The nearly invariant leukaemogenesis of E_{μ} -*myc* mice might be taken as evidence that an active *myc* gene is sufficient for lymphoid neoplasia, but several observations make us think that other events (presumably genetic) influence tumour development. First, the latent period before any overt signs arise is variable and can be as long as 5 months, whereas we would expect E_{μ} -driven *myc* expression to commence concomitantly with B-cell ontogeny, well before birth. Second, there appears to be a true pre-neoplastic state in these mice, since no malignant cells, as assessed by transplantation, are detectable within the lymphoid organs of young transgenic mice free of overt disease (A.W.H., unpublished results). Finally, we are struck by the fact that many of the tumours are monoclonal (Table 1). Since the E_{μ} -*myc* gene should be active within the entire B-lymphoid compartment, clonality implies that one lymphocyte acquired a decided growth advantage, probably by some still undefined genetic alteration. Conceivably the background of the mice (C57BL \times SJL) F_2 might also have some role, since aged SJL mice are subject to a neoplastic disorder of the lympho-reticular system⁵¹.

If dysregulated *c-myc* expression is insufficient for malignancy, why does it elicit high-frequency tumorigenesis? *myc* expression is normally associated with dividing cells and drops precipitously as the cell enters the resting (G_0) state¹⁸⁻²⁰. Thus, constitutive *myc* expression above a certain threshold may promote cell division at the expense of differentiation. The probability that a given cell will proliferate (self-renew) rather than generate differentiated progeny may be set by the level of *c-myc*, or a balance between *myc* and differentiation-promoting elements. This model predicts that excess *myc* expression in a given

lineage will increase the proportion of cells that are less mature and therefore have greater proliferative capacity. Any accidental event that further increases the proliferation/differentiation ratio in a member of this large vulnerable population then creates the malignant clone.

It already seems clear that the diverse set of B-lymphoid cell lines generated by E_{μ} -myc mice will aid studies on lymphoid ontogeny and the mechanisms that regulate immunoglobulin gene rearrangement and expression. Even more significant, the very high predisposition of these mice to neoplasia provides new opportunities for exploring the pre-neoplastic state. It will be intriguing to discover in what ways the haematopoietic popu-

lations have been perturbed by this cellular oncogene freed from its normal restraints.

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Role of acetylcholine receptor subunits in gating of the channel

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The Torpedo and calf acetylcholine receptors and hybrids composed of subunits from the two species have been produced in Xenopus oocytes by the use of the cloned complementary DNAs. Single-channel current measurements indicate that these receptors form channels of similar conductance but with different gating behaviour.

THE nicotinic acetylcholine receptor (AChR) is a transmembrane protein constituting a ligand-gated ionic channel. In the absence of acetylcholine (ACh), the channel is in the closed state. When ACh is bound to the receptor, the channel opens for a few milliseconds, giving rise to an elementary current pulse¹, the size and duration of which can be observed directly by the patch-clamp technique². Biochemical evidence has shown that the AChR from *Torpedo* electroplax consists of four kinds of homologous subunits assembled in a molar stoichiometry of $\alpha_2\beta\gamma\delta$ and that the α -subunit carries the ACh-binding site³⁻⁵. The primary structures of all four subunits of the *Torpedo* electroplax⁶⁻¹¹ and mammalian muscle AChRs¹²⁻¹⁷, the γ - and δ -subunits of the avian AChR¹⁸ and the newly found ϵ -subunit of the calf muscle AChR¹⁹ have been elucidated by cloning and

sequencing complementary DNAs or genomic DNAs encoding these polypeptides. The cloned cDNAs encoding the α -, β -, γ - and δ -subunits of the *Torpedo* AChR can be expressed to produce the functional receptor in *Xenopus* oocytes^{20,21} and analysis of the functional properties of AChR mutants possessing α -subunits altered by site-directed mutagenesis of the cDNA has enabled functional regions of the subunit molecule to be localized²¹.

One possible approach to studying the functional roles of the individual subunits in ion transport and gating of the AChR channel would be to construct hybrid AChR molecules using subunits from different species and to compare their channel properties with those of the parental AChRs. It has been shown recently that the hybrid AChRs composed of the *Torpedo* α -,

Table 1 Functional properties of *Torpedo*, calf and hybrid AChRs

Combination of subunit-specific mRNAs	¹²⁵ I- α -BTX binding (fmol per oocyte)		Whole-cell current				Single-channel current	
	Surface	Extract	AChR channel activity (nA)	Reversal potential (mV)	Voltage sensitivity	Hill coefficient	Conductance (pS)	Average duration (ms)
$\alpha_T\beta_T\gamma_T\delta_T$	7.6 \pm 2.6 (4)	33.7 \pm 19.1 (4)	23 \pm 10 (8)	-4.0 \pm 5.8 (8)	1.0 \pm 0.05 (4)	1.7 \pm 0.2 (4)	42 \pm 2 (5)	0.6 \pm 0.2 (3)
$\alpha_C\beta_C\gamma_C\delta_C$	7.9 \pm 2.6 (4)	84.9 \pm 32.9 (4)	1,600 \pm 1,250 (10)	-11.0 \pm 2.9 (3)	3.1* (2)	1.6† (2)	40 \pm 0.3 (3)	7.6 \pm 1.4 (4)
$\alpha_C\beta_T\gamma_T\delta_T$	10.4 \pm 3.0 (4)	104.2 \pm 36.0 (4)	80 \pm 45 (6)	-6.9 \pm 2.9 (5)	1.0 \pm 0.05 (3)	1.8 (1)	42 \pm 2 (3)	2.5 \pm 0.6 (4)
$\alpha_T\beta_C\gamma_T\delta_T$	0.1 \pm 0.1 (4)	9.1 \pm 5.2 (4)	<1 (12)	—	—	—	—	—
$\alpha_T\beta_T\gamma_C\delta_T$	0.3 \pm 0.2 (4)	9.5 \pm 6.7 (4)	<1 (5)	—	—	—	—	—
$\alpha_T\beta_T\gamma_T\delta_C$	5.4 \pm 3.0 (4)	15.2 \pm 5.1 (4)	1,400 \pm 490 (9)	-1.4 \pm 2.5 (8)	2.0 \pm 0.4 (6)	1.7‡ (2)	35 \pm 3 (3)	8.6 \pm 1.3 (3)

Xenopus laevis oocytes were injected with various combinations of *Torpedo* (indicated by the suffix T) and calf (indicated by the suffix C) AChR subunit-specific mRNAs as specified; molar ratio of the α -, β -, γ - and δ -subunit-specific mRNAs, 2:1:1:1; total mRNA concentration, 100–140 ng μ l⁻¹; average volume injected per oocyte, 20–50 nl. The oocytes were incubated for 2–4 days in conditions described previously²⁰ before being tested for α -BTX binding activity or response to ACh. Data are given as means \pm s.d. Numbers in parentheses refer to the number of experiments for α -BTX binding activity (pools of 10–20 oocytes used in each experiment) or the number of oocytes or membrane patches for current measurements. For assay of the toxin-binding activity on the cell surface³², intact oocytes were incubated at room temperature for 3 h with 2 nM ¹²⁵I- α -BTX (213 Ci mmol⁻¹) in modified Barth's medium³⁴ containing 1 mg ml⁻¹ bovine serum albumin. The toxin-binding activity in cell extracts was determined by the antibody precipitation assay as described previously^{20,21}, using the monoclonal antibody reactive with the calf AChR (mAb210) for oocytes injected with the subunit-specific mRNA combination $\alpha_C\beta_C\gamma_C\delta_C$ or $\alpha_C\beta_T\gamma_T\delta_T$, or rabbit antiserum to the *T. californica* AChR for oocytes injected with the other mRNA combinations; incubation was at room temperature for 3 h in the presence of 2 nM ¹²⁵I- α -BTX. AChR channel activity represents membrane currents in response to bath application of 0.1 μ M ACh. The membrane potential was voltage-clamped at -70 mV. Oocytes injected with the subunit-specific mRNA combination $\alpha_T\beta_C\gamma_T\delta_T$ or $\alpha_T\beta_T\gamma_C\delta_T$ did not respond to 0.1 μ M ACh, but they responded to 0.5 μ M ACh, the average channel activity being 18 \pm 22 nA(7) and 5 \pm 3 nA(3), respectively. Reversal potentials were determined as described in Fig. 1 legend. The voltage sensitivity was quantified as the ratio of the slope conductances at -100 mV and 0 mV membrane potential, measured in Ringer's solution with 1.0 mM or 1.8 mM Mg²⁺ replacing Ca²⁺ (ref. 21). Conductance values represent slope conductances. Mean current amplitudes were measured at least at three different membrane potentials, ranging from -120 mV to -20 mV, in each experiment. The single-channel conductance was obtained as the slope of the *i*-*V* relation determined by linear regression. The average durations of single channel currents are defined as follows. Single-channel current burst durations were measured as any sequence of events separated by less than a critical closed interval of 1 ms. The distributions of such bursts were fitted satisfactorily only by the sum of two exponential components, except for some measurements of the *Torpedo* AChR (see text). The average current durations given represent the slower component. In all cases, the slower component represented more than 70% of the area of the distribution. Averages are from experiments at -110 mV to -70 mV membrane potential.

* Mean of 2.8 and 3.4; † mean of 1.45 and 1.78; ‡ mean of 1.55 and 1.94.

β - and δ -subunits and either the calf γ - or ϵ -subunit are functional when expressed in *Xenopus* oocytes¹⁹. Here we report conductance and gating properties of the *Torpedo* and calf AChRs as well as of the hybrid AChRs in which the α - or δ -subunit of the *Torpedo* AChR is replaced by the corresponding subunit of the calf AChR. Substitution of the calf δ -subunit in the *Torpedo* AChR alters the gating behaviour of the channel drastically, making it similar to the calf AChR channel. Substitution of the calf α -subunit has less effect on the gating properties of the *Torpedo* AChR channel. The conductance of the channel is not significantly affected by either substitution.

Torpedo and calf AChR channels

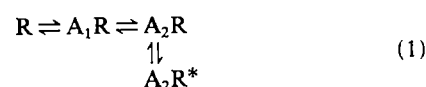
Activity of AChR channels in oocytes. The mRNAs specific for the α -, β -, γ - and δ -subunits of the *Torpedo californica* electroplax and calf muscle AChRs were synthesized *in vitro*^{22,23} using the respective cDNA templates (see refs 19, 21 and Fig. 1 legend). The four subunit-specific mRNAs for the *Torpedo* or calf AChR were combined and introduced into *Xenopus* oocytes by microinjection. The oocytes injected with the calf mRNAs responded to ACh much more strongly than did the oocytes injected with the *Torpedo* mRNAs (Fig. 1a, b), the average membrane currents activated by 0.1 μ M ACh being 1,600 nA and 23 nA, respectively (Table 1). This large difference in membrane current cannot be accounted for by different densities of the calf and *Torpedo* AChRs expressed in the oocyte membrane because the binding activity of α -bungarotoxin (α -BTX) on the cell surface did not differ significantly between the two groups of oocytes (Table 1). These observations imply some functional differences between the calf and *Torpedo* AChR channels.

The current through a membrane (I_m) is given by $I_m = n i p(o)$, where n represents the number of channels, i the current through a single channel at a given membrane voltage, and $p(o)$ the probability of the channel being open. Because it can be assumed that the number of channels does not vary significantly between the two groups of oocytes, the difference in channel activity

could reflect a larger elementary current flowing through a single calf AChR channel, or it could reflect a higher open probability of the channel at the given ACh concentration. The following whole-cell and single-channel current measurements were designed to decide between these possibilities.

Figure 1c, d shows current-voltage (*I*-*V*) relations of the ACh-activated current determined in oocytes injected with either the four *Torpedo* or the four calf subunit-specific mRNAs. The reversal potential of the ACh-activated current was -11 to -4 mV in both groups of oocytes (Table 1), suggesting that both the *Torpedo* and the calf AChR channels have similar ion selectivity. A marked difference was observed, however, in the shape of the *I*-*V* relation. In oocytes implanted with the *Torpedo* AChR, the *I*-*V* relation was almost linear, but was curved for oocytes implanted with the calf AChR, indicating an inward rectifying conductance similar to that of frog endplate channels^{24,25}.

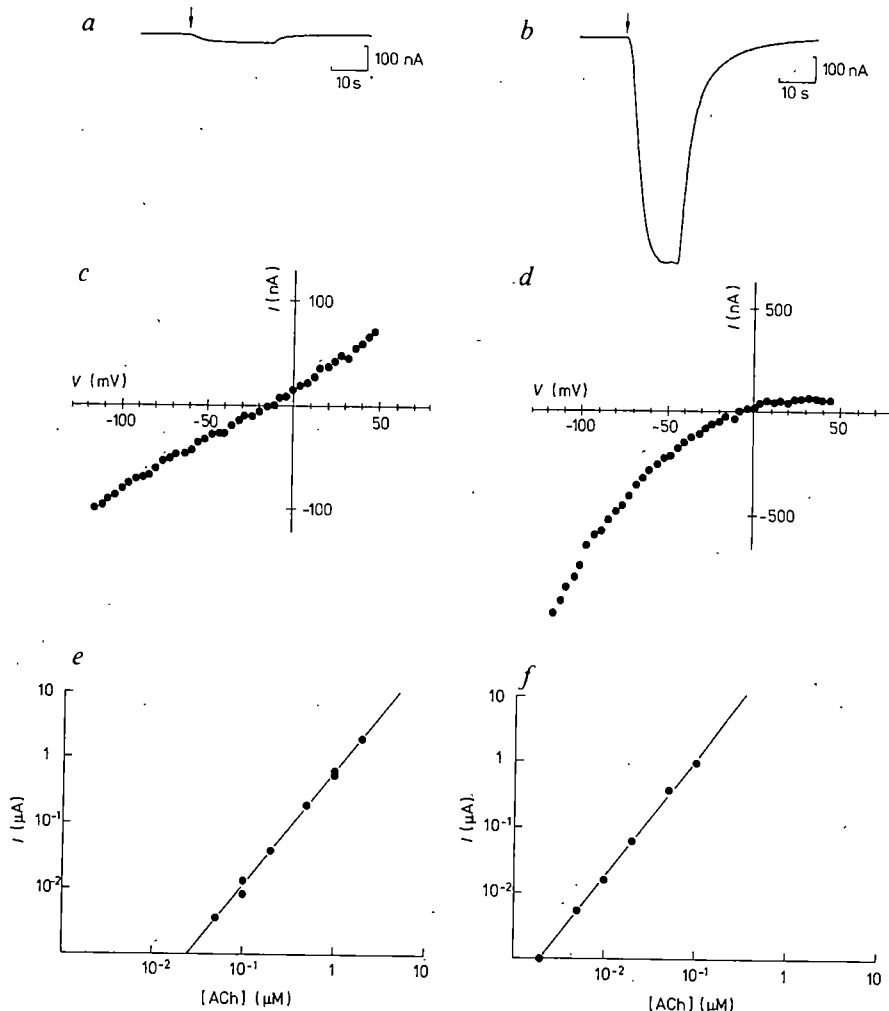
The relation between ACh concentration and membrane current was supralinear, with a Hill coefficient of 1.6–1.7 for both groups of oocytes (Fig. 1e, f, Table 1). This indicates that the *Torpedo* and calf AChRs are comparable in stoichiometry of ACh binding and that the large difference in oocyte channel activity is probably not due to a difference in agonist-receptor stoichiometry. A plausible reaction mechanism for the opening of AChR channels in muscle assumes that channel opening occurs from the bi-liganded receptor²⁶. The agonist-receptor interaction is described by



where R is the resting receptor, A_1R and A_2R are the mono-liganded and bi-liganded receptors, respectively, and the asterisk indicates the open channel state. This simplified reaction scheme does not take other reaction pathways into account. However, it can describe the supralinear relation between ACh concentra-

Fig. 1 ACh-activated whole-cell currents in oocytes injected with the α -, β -, γ - and δ -subunit-specific mRNAs for *Torpedo* AChR (a, c, e) or calf AChR (b, d, f). a, b, Whole-cell current activated by 0.1 μ M ACh, bath-applied at the time indicated by the arrow. Inward current is downwards. Membrane potential, -70 mV. The current amplitude is 30 nA (a) or 960 nA (b). c, d, *I*-*V* relation of ACh-activated current. Bath application of 0.5 μ M (c) or 0.1 μ M (d) ACh. e, f, Relation between concentration of ACh and amplitude of whole-cell current response. Double logarithmic coordinates. Linear regression yields a slope of 1.7 (e) or 1.8 (f).

Methods. The pSP65 (ref. 23) recombinants carrying the calf AChR α -, β - or δ -subunit cDNA were constructed as follows. The *Sau96I*(-83)/*RsaI*(57) fragment from pcACR α 40 (ref. 12) and the *RsaI*(57)/*PstI*(108) fragment from pcACR α 18 (ref. 12) were ligated. The resulting 190-base-pair (bp) fragment was ligated with the 3.0-kilobase-pair (kb) *PstI*/*HincII* fragment from pSP65. The ligated DNA fragments were treated with *Escherichia coli* DNA polymerase I (Klenow fragment) in the presence of dGTP and dCTP and ligated. A plasmid (pc α SP1) carrying the nucleotide residues -83 to 107 of the calf AChR α -subunit cDNA was identified by colony hybridization with the above *Sau96I*/*RsaI* fragment and by restriction endonuclease analysis. The *PstI*(108)/*PstI*(428) fragment from pcACR α 18 was inserted into the *PstI* site of pc α SP1 in the same orientation as the SP6 promoter. The 366-bp *EcoRI*/*BglII* fragment from the resulting plasmid, the *BglII*(252)/*XbaI*(1,755) fragment from pcACR α 18 and the 3.0-kb *XbaI*/*EcoRI* fragment from pSP65 were ligated to yield pc α SP2. The 1.9-kb fragment from pcACR α 18 extending from the *XbaI*(1,755) site to the *PvuII* site on the vector DNA, and the 3.0-kb *SmaI*/*XbaI* fragment from pSP64 were ligated. The 1.9-kb *XbaI*/*SacI* fragment from the resulting plasmid, the 1.9-kb *EcoRI*/*XbaI* fragment from pc α SP2 and the 3.0-kb *SacI*/*EcoRI* fragment from pSP65 were ligated to yield pSP α . The 308-bp *AvaII* fragment from pcACR β 325¹³ was treated with Klenow fragment in the presence of dGTP, dCTP and dTTP and cleaved with *AccI*. The resulting 268-bp fragment was ligated with the *AccI*(162)/*BamHI*(598) fragment from pcCR β 110¹³ and the 3.0-kb *BamHI*/*SmaI* fragment from pSP65 to yield pc β SP1. The *BamHI*(598)/*BanI*(1,781) fragment and the 704-bp fragment extending from the *BanI*(1,781) site to the *PvuII* site on the vector DNA, derived from clone pcACR β 110, were ligated with the 3.0-kb *HincII*/*BamHI* fragment from pSP65 to yield pc β SP2. The 719-bp *EcoRI*/*BamHI* fragment from pc β SP1 and the 4.9-kb *BamHI*/*EcoRI* fragment from pc β SP2 were ligated to yield pSP β . The ~290-bp *PstI* fragment from pcACR δ 315¹⁵ was inserted into the *PstI* site of pSP65 in the reverse orientation as the SP6 promoter. This plasmid was cleaved with *HindIII*, treated with *Bal31* exonuclease and cleaved with *PstI*. The resulting ~260-bp fragments were ligated with the 3.0-kb *PstI*/*SmaI* fragment from pSP65. A plasmid (pc δ SP1) carrying a *SmaI* site immediately before the initiation codon was identified by DNA sequencing³³. The ~350-bp *PstI* fragment from pcACR δ 315 was inserted into the *PstI* site of pc δ SP1 in the same orientation as the SP6 promoter to yield pc δ SP2. The ~370-bp fragment from pcACR δ 496¹⁵, extending from *XbaI*(1,485) site to the *PvuII* site on the vector DNA, was ligated with the 3.0-kb *HincII*/*XbaI* fragment from pSP65 to yield pc δ SP3. The 3.3-kb *XbaI*/*EcoRI* fragment from pc δ SP3, the 431-bp *EcoRI*/*AccI* fragment from pc δ SP2 and the *AccI*(351)/*XbaI*(1,485) fragment from pcACR δ 496 were ligated to yield pSP δ . The calf AChR α -, β - and δ -subunit-specific mRNAs were synthesized *in vitro*^{22,23} using pSP α , pSP β and pSP δ (each cleaved with *HindIII*), respectively, and were capped²². The calf AChR γ -subunit-specific mRNA and the *T. californica* AChR α -, β -, γ - and δ -subunit-specific mRNAs were synthesized as described previously^{19,21}. Microinjection and incubation of oocytes were carried out as described for Table 1. All electrophysiological measurements were performed at 19–21 °C in frog Ringer's solution of the following composition (in mM) unless otherwise specified: NaCl, 115; KCl, 2.5; CaCl₂, 1.8; HEPES (pH 7.2), 10. Atropine (0.5 μ M) was added to all bath solutions to block muscarinic AChRs. Follicular cells were removed from oocytes by treatment with 1 mg ml⁻¹ collagenase (Sigma type I) in modified Barth's medium³⁴ at room temperature for 1 h. Oocytes were then held in an experimental chamber of 0.5 ml volume through which Ringer's solution was perfused continuously at 5 ml s⁻¹. Oocytes were voltage-clamped with a conventional two-micropipette voltage clamp. The voltage-recording pipette was filled with 3 M KCl, and the current injection pipette with a solution containing 0.25 M CsF, 0.25 M CsCl and 50 mM EGTA. ACh-containing solution was perfused for 10 s through the chamber. *I*-*V* relations were measured by changing the membrane potential in a rampwise fashion (40 mV s⁻¹) from -120 mV to +50 mV in the absence and presence of low (<1 μ M) ACh concentrations. The difference current was obtained by subtracting the two current records at 4-mV intervals. The reversal potential was obtained by linear interpolation between inward and outward current values measured close (<12 mV) to the reversal potential. All *I*-*V* relations were determined in extracellular solution where 1.0 mM or 1.8 mM Mg²⁺ replaced Ca²⁺ (ref. 21). The relation between ACh concentration and current amplitude was measured at -70 mV in normal Ringer's solution. ACh (2 nM–2 μ M) was bath-applied for 10 s.



tion and membrane current observed for AChR-implanted oocytes.

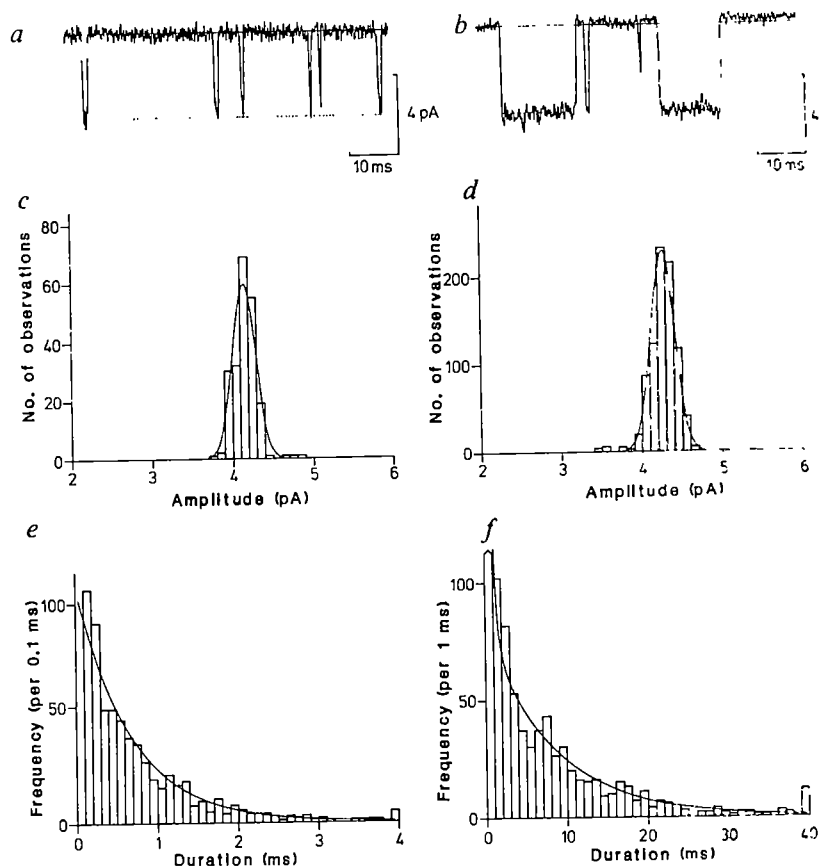
Elementary currents. Figure 2a,b compares ACh-activated single-channel currents recorded from outside-out patches isolated from oocytes injected with either the four *Torpedo* or the four calf subunit-specific mRNAs. In the two patches, the amplitudes of single-channel currents at -100 mV membrane potential were of similar size, that is, 4.1 pA and 4.2 pA for the *Torpedo* and calf AChR channels, respectively (Fig. 2c,d). The single-channel *i*-*V* relations were linear, both channels exhibiting a slope conductance close to 40 pS (Table 1).

To characterize the ion selectivity of the two classes of AChR channels, we determined the conductance sequence for

monovalent metal cations. In nominally Ca²⁺-free extracellular solution (10 mM EGTA added), the conductance sequence of the *Torpedo* and calf AChR channels was K⁺ > Cs⁺ > Na⁺ > Li⁺. The single-channel slope conductances for these cations, determined at an extracellular cation concentration of 100 mM and a driving potential of -100 mV, were (mean of two patches): 107 pS, 90 pS, 60 pS and 30 pS for the *Torpedo* AChR and 70 pS, 60 pS, 50 pS and 21 pS for the calf AChR. These values are close to the limiting conductances because the conductance did not increase further when the extracellular cation concentration was raised to 200 mM or 300 mM. The half-saturation concentrations were in the range 20–30 mM. The conductance sequence corresponds to the Eisenman sequence III or IV (ref. 27), which

Fig. 2 Properties of ACh-activated single-channel currents recorded from outside-out patches isolated from oocytes implanted with *Torpedo* AChR (*a, c, e*) or calf AChR (*b, d, f*). *a, b*, Records of single-channel currents. Inward current is downwards. Membrane potential, -100 mV. Low-pass filtering at 3 kHz (*a*) or 2 kHz (*b*) (-3 dB). At these filter settings the short interruptions of elementary currents occurring in the time range of tens of microseconds^{31,32} are largely attenuated in the records shown. *c, d*, Distribution of current amplitudes. Membrane potential, -100 mV; mean amplitude, 4.1 ± 0.14 pA (*c*) or 4.2 ± 0.15 pA (*d*) (mean \pm s.d.). *e, f*, Distribution of current durations. Membrane potential, -100 mV. The distribution in *e* is fitted by a single exponential; decay time constant, 0.61 ms. The distribution in *f* is fitted by the sum of two exponentials; decay time constants, 0.4 and 8.6 ms. Only the slower component is shown fully in *f*. The last bin includes durations longer than 4 ms (*e*) or 40 ms (*f*). The first bin in *f* has a roof to indicate that it extends beyond the scale.

Methods. Single-channel currents were measured in outside-out and cell-attached membrane patches³⁵. All experiments were done at 19 – 21 °C on oocytes bathed in normal frog Ringer's solution. The vitelline membrane covering the oocyte was removed mechanically to allow close contact between pipette and oocyte plasma membrane with a seal resistance >10 G Ω . Mechanical removal of the vitelline membrane was facilitated by placing the oocyte for 5 min in hypertonic 'stripping solution' (in mM: K-aspartate 200; KCl 20; MgCl₂ 1; EGTA 10; HEPES (pH 7.2) 10). The pipette solution in the outside-out patch recording configuration had the following composition (in mM): KCl 20; KF 80; MgCl₂ 1; EGTA 10; HEPES (pH 7.2) 10. Following formation of an outside-out patch, ACh was applied from another pipette the opening of which (≈ 500 μ m diameter) was located close to the membrane patch (~ 100 μ m distance). The ACh concentration in this pipette was 0.1 – 5 μ M. The conductance sequences for different alkali metal cations were measured in cell-attached patches. Thick-walled patch pipettes of small (≈ 0.5 μ m diameter) tip openings were used. With these pipettes the occurrence of endogenous stretch-activated single-channel currents is significantly reduced (C.M. *et al.*, in preparation). K⁺, Cs⁺, Na⁺ or Li⁺ were present at 100 mM in the pipette solution which contained also 10 mM EGTA and 10 mM HEPES (pH 7.2). Conductance values represent the slope conductance measured at a driving potential of -100 mV. Patch-clamp currents were measured with a LIST EPC-7 amplifier and recorded on magnetic tape (Racal Store 4). After appropriate filtering, current records were digitized at 20–100 μ s per point and analysed on a PDP 11/73 computer. For analysis of the amplitude and duration of single-channel current records of the *Torpedo* AChR, the method of time course fitting³⁶ was used. Currents mediated by the calf and hybrid AChRs were analysed also by the threshold-crossing method³⁶; the threshold was half the full amplitude. Amplitude distributions contained only fully resolved current pulses and were fitted by single Gaussians. Single channel current *i*–*V* relations in outside-out patches were measured in the voltage range -120 to -20 mV. Distributions of durations were fitted with either one or two exponential components using the maximum likelihood method³⁶. The Patternsearch or the Simplex method was used to obtain the parameters of the distribution³⁶. The average duration of elementary currents in the text and in Table 1 refers to the decay time constant of the slower component.



suggests, in terms of Eisenman's selectivity theory, a relatively weak interaction between the ion in the channel and an anionic binding site on the channel wall in both *Torpedo* and calf AChR.

Whereas the ion selectivity and transport properties of the *Torpedo* and calf AChR channels were comparable, their gating behaviour differed markedly. The average duration of elementary currents flowing through calf AChR channels was much longer than that observed for the *Torpedo* AChR channel. Figure 2*e, f* shows histograms of current durations at -100 mV membrane potential. Distributions of current durations were fitted satisfactorily only by the sum of two exponential components, except for some measurements of the *Torpedo* AChR channel, where the number of resolved short durations was too small to allow a reliable estimate of the decay time constant of the faster component. The slower component predominated in all experiments and we therefore restricted the analysis to this component. The average duration of single-channel currents was 0.6 ms for *Torpedo* AChR at membrane potentials of -110 mV to -70 mV, in striking contrast to the 7.6 ms obtained for calf AChR (Table 1).

Another difference between the two AChR channels was revealed when the average duration of elementary currents was measured at different membrane potentials. The average current duration of the *Torpedo* AChR channel was not significantly

voltage-dependent; in one experiment, measured at membrane potentials of -50 mV and $+50$ mV in the same membrane patch, they were 0.48 and 0.46 ms, respectively. Elementary currents of the calf AChR channel (Fig. 2*f*) had average durations of 8.6 ms, 6.8 ms and 3.9 ms at membrane potentials of -100 mV, -50 mV and $+50$ mV, respectively, suggesting that the gating reaction of the calf AChR is voltage-dependent. This probably underlies the inward rectification in the whole-cell *I*–*V* relation for the calf AChR (see Fig. 1*d*).

Hybrid AChR channels

To determine which subunit is responsible for the marked difference in gating behaviour between the *Torpedo* and calf AChRs, we constructed hybrid AChRs by injecting oocytes with the corresponding combinations of *Torpedo* and calf subunit-specific mRNAs, then analysed the functional properties of the hybrid AChRs.

α -Hybrid AChR. The hybrid AChR formed in oocytes injected with the calf α -subunit-specific mRNA and the *Torpedo* β -, γ - and δ -subunit-specific mRNAs (α -hybrid AChR) showed an average channel activity somewhat higher than that of the *Torpedo* AChR but much lower than that of the calf AChR (Fig. 3*a*, Table 1). The whole-cell *I*–*V* relation was almost linear, and the reversal potential close to 0 mV (Fig. 3*b*, Table 1); the dose-

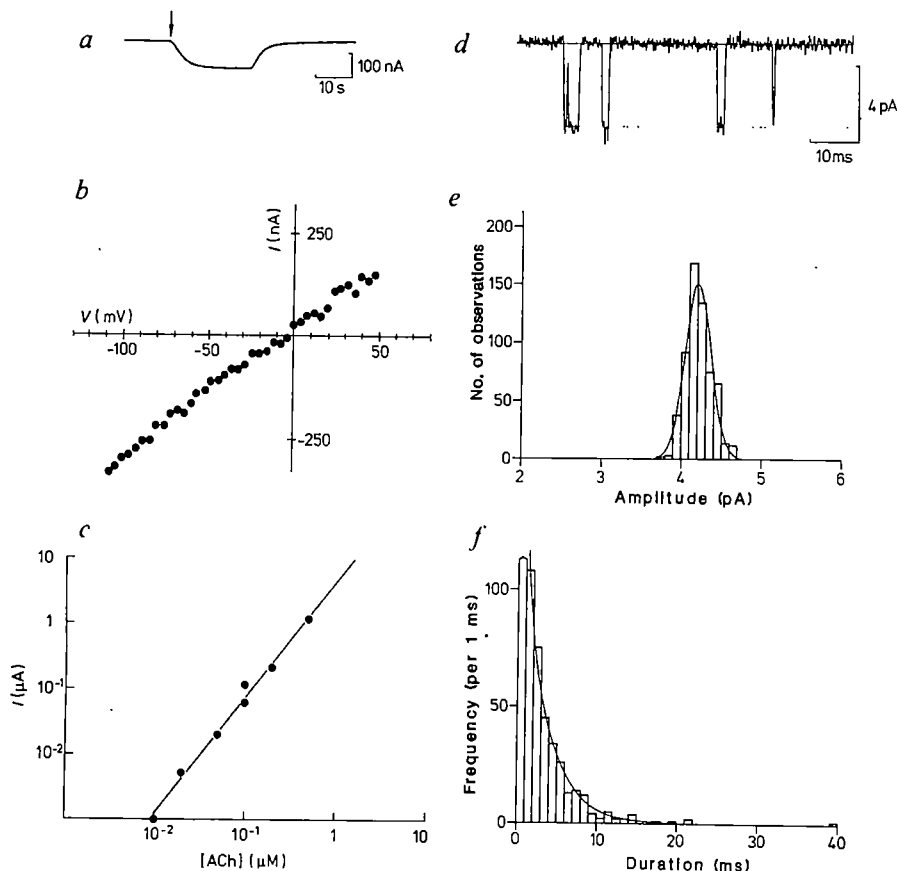


Fig. 3 Whole-cell (*a-c*) and single-channel (*d-f*) current measurements for oocytes implanted with the α -hybrid AChR. *a*, Whole-cell current activated by $0.1 \mu\text{M}$ ACh bath-applied at the time indicated by the arrow. Inward current is downwards. Membrane potential, -70 mV . The current amplitude is 120 nA . Note that the scales are the same as in Fig. 1*a*. *b*, *I-V* relation of ACh-activated current between -120 mV and $+50 \text{ mV}$ membrane potential. *c*, Relation between ACh concentration and whole-cell current response. Double logarithmic coordinates. The slope of the straight line fit is 1.8 . *d*, Single-channel currents. Inward current is downwards. Membrane potential, -100 mV . Low-pass filtering at 2 kHz (-3 dB). *e*, Distribution of current amplitudes. Membrane potential, -100 mV . The mean amplitude is $4.2 \pm 0.16 \text{ pA}$. *f*, Distribution of current durations. Membrane potential, -100 mV . Line represents fit by the sum of two components; decay time constants, 0.41 ms and 3.2 ms . Only the slower component is shown fully. The last bin includes durations $>40 \text{ ms}$.

response curve yielded a Hill coefficient of 1.8 (Fig. 3*c*). The properties of whole-cell currents (Table 1) indicate that the α -hybrid AChR behaves much like the *Torpedo* AChR.

Figure 3*d* shows single-channel current recordings of the α -hybrid AChR, and Fig. 3*e, f* the distribution of current amplitudes and of current durations at -100 mV membrane potential. The channel conductance of this hybrid AChR (42 pS) was virtually the same as that of the parental AChRs (Table 1). The mean duration of single-channel currents of this hybrid (2.5 ms) was longer than that of the *Torpedo* AChR (Table 1). The average duration of single-channel currents of the α -hybrid AChR was essentially independent of membrane potential; the values obtained at membrane potentials of -100 mV and $+50 \text{ mV}$ were 3.2 and 2.7 ms , respectively (Fig. 3*f*).

δ -Hybrid AChR. The hybrid AChR formed in oocytes injected with the calf δ -subunit-specific mRNA and the *Torpedo* α -, β - and γ -subunit-specific mRNAs (δ -hybrid AChR) showed an average channel activity ~ 60 -fold higher than that of the *Torpedo* AChR and comparable to that of the calf AChR (Fig. 4*a*, Table 1). The whole-cell *I-V* relation was curved, indicating that the ACh-activated conductance mediated by the δ -hybrid AChR channel rectifies and the reversal potential was close to 0 mV (Fig. 4*b*, Table 1). The dose-response curve gave a Hill coefficient of 1.7 (Fig. 4*c*, Table 1).

Single-channel current records (Fig. 4*d*) revealed that the channel conductance and gating behaviour of the δ -hybrid AChR are similar to those of the calf AChR. Figure 4*e* and *f* show the distribution of current amplitudes and of current durations at -110 mV membrane potential, respectively. The channel conductance of the δ -hybrid AChR (35 pS) was nearly the same as that of the parental AChRs (Table 1). However, the average duration of elementary currents was 8.6 ms , more than 10 -fold longer than that of the *Torpedo* AChR and comparable to that of the calf AChR (Table 1). The average current duration of the δ -hybrid AChR channel was voltage-dependent being 10.3 ms and 3.5 ms at membrane potentials of -110 mV and $+50 \text{ mV}$, respectively (Fig. 4*f*).

β - And γ -hybrid AChRs. Two additional hybrid AChRs were produced by injecting oocytes either with the calf β -subunit-specific mRNA and the *Torpedo* α -, γ - and δ -subunit-specific mRNAs (β -hybrid AChR) or with the calf γ -subunit-specific mRNA and the *Torpedo* α -, β - and δ -subunit-specific mRNAs (γ -hybrid AChR). Oocytes containing these hybrid AChRs showed a very low channel activity (Table 1). This is probably due to an extremely low density of the β - and γ -hybrid AChRs expressed in the oocyte membrane because the α -BTX binding activity on the cell surface was markedly diminished despite the considerable activity found in the cell extract (Table 1). It thus appears that in these hybrids subunit assembly is impaired, forming aberrant complexes. Therefore, we did not investigate channel properties of the β - and γ -hybrid AChRs.

Conclusions

Both *Torpedo* and calf AChR channels, produced in the *Xenopus* oocyte membrane by translation of the α -, β -, γ - and δ -subunit-specific mRNAs, exhibit virtually the same conductance. In Ringer's solution, with Na^+ as the main charge carrier, the conductance is $\sim 40 \text{ pS}$. Any subunit of the *Torpedo* AChR can be replaced by the corresponding subunit of the calf AChR to form functional channels. The conductances of α - and δ -hybrid AChR channels are similar to those of the parental AChR channels. These observations are consistent with the high degree of amino-acid sequence homology between corresponding subunits of the *Torpedo* and calf AChRs⁶⁻¹⁵. Each subunit contains five putative transmembrane segments^{6-9,11-15,21,28,29} which are involved directly in the formation of the channel²¹.

In contrast to the uniform conductance of the *Torpedo*, calf and hybrid AChR channels, their gating behaviour differs widely. The average duration of elementary currents of the calf AChR channel is more than 10 -fold that of the *Torpedo* AChR channel. The mean current duration of the α -hybrid AChR channel lies between those of the *Torpedo* and calf AChR channels, while that of currents through the δ -hybrid AChR channel is comparable to that of the calf AChR channel. There-

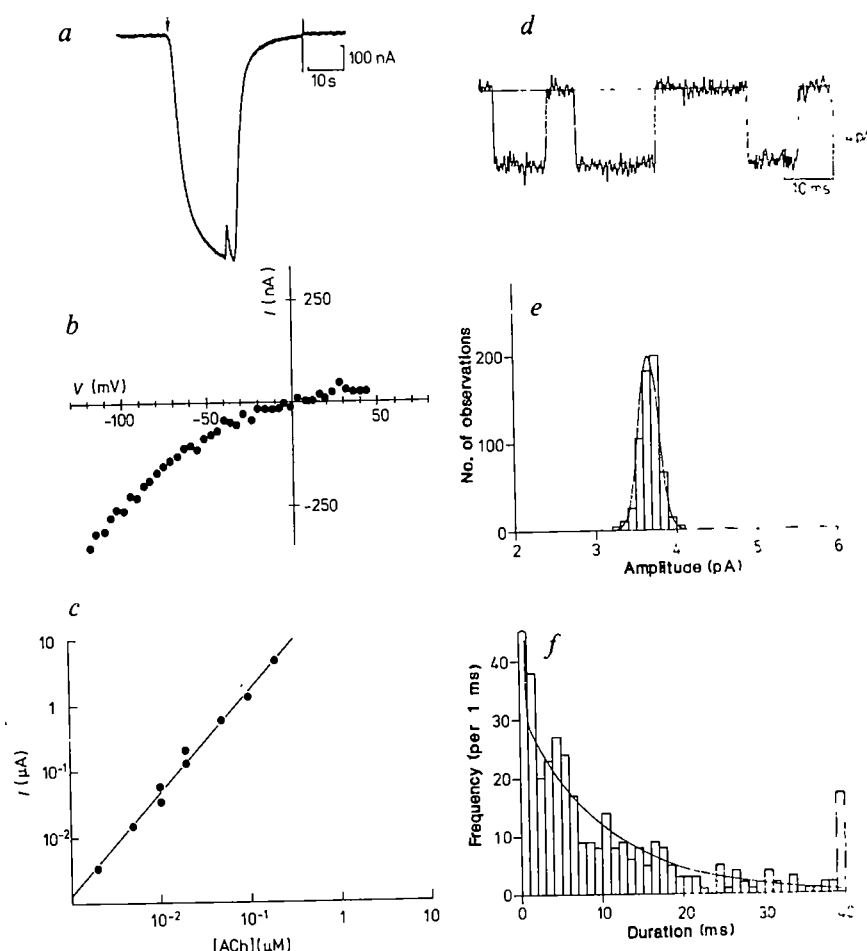


Fig. 4 Whole-cell (*a-c*) and single-channel (*d-f*) current measurements for oocytes implanted with the δ -hybrid AChR. The experimental conditions were the same as for Fig. 3, unless otherwise stated. *a*, ACh-activated whole-cell current. Current amplitude, 940 nA. *b*, $I-V$ relation of ACh-activated current. *c*, Relation between ACh concentration and whole-cell current response. The slope of the straight line fit is 1.6. *d*, Record of single-channel currents. Membrane potential, -110 mV. *e*, Distribution of current amplitudes. Membrane potential, -110 mV. The mean amplitude is 3.7 ± 0.12 pA. *f*, Distribution of current durations. Membrane potential, -110 mV. Decay time constants, 0.14 ms and 10.3 ms.

fore, it seems plausible that presently unidentified functional domains of the calf δ - and α -subunits determine the longer duration of elementary currents.

The average duration of elementary currents through AChR channels is determined, in the framework of the simple two-step reaction mechanism (1) described above, by at least three reaction steps: channel opening, channel closing and dissociation of the agonist. The increased duration of elementary currents in the δ -hybrid and α -hybrid AChR channels, as compared with that of the *Torpedo* AChR channel, could reflect a difference in any of the rate constants governing these steps. The voltage dependence of whole-cell $I-V$ relations and single-channel current durations might give a clue as to which reaction step is different. In frog endplate, the curvature of the whole-cell $I-V$ relation and the voltage sensitivity of the average single-channel current duration are determined by the voltage dependence of

channel closing^{30,31}. The observation that both the calf AChR and the δ -hybrid AChR behave like the frog AChR would then be consistent with the hypothesis that the δ -subunit determines the channel-closing step. The α -hybrid AChR is also characterized by an average current duration somewhat longer than that of the *Torpedo* AChR but which is voltage-insensitive, suggesting that the α -subunit may mainly determine another reaction step, such as channel opening or agonist dissociation.

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Shepherding model for Neptune's arc ring

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An incomplete arc ring has recently been discovered around Neptune¹. Here, a model to explain the confinement of this ring is developed. The ring may be azimuthally confined near a triangular (Trojan) point of an undiscovered satellite of Neptune. Radial diffusion of the ring particles can be prevented by shepherding torques of another moon. Two satellites with diameters of 100–200 km would be sufficient to confine the ring; such moons would be too small to have been photographed from Earth.

The arc ring of Neptune¹ differs substantially from other known planetary rings. Whereas the rings of Jupiter, Saturn and Uranus completely encircle these planets, Neptune's ring appears to be an incomplete arc, spanning only a small fraction of an ellipse. The exact dimensions of the ring are poorly constrained, as it has only been observed by stellar occultation. Detections of the ring at three separate telescopes imply that it is located at a distance of $r \sim 67,000$ km from Neptune's centre, has a width $w \sim 15$ km, an optical depth $\tau \sim 0.08$, and a length of at least 100 km, all assuming that the ring lies in Neptune's equatorial plane¹. Approximately 10 other stellar occultations of the Neptune system have failed to show such a ring, although an event observed at a similar distance from Neptune by two closely spaced telescopes during one occultation, which has been tentatively attributed to a satellite (provisionally named 1981N1)², could possibly be an optically very thick ring. The current detection rate leads to a crude estimate that the arc ring spans $\sim 10\%$ of the circumference around Neptune.

Theories developed to explain the rings of other planets do not fully explain arc rings. If the particles in an arc ring were unconstrained, they would spread out azimuthally because of the shorter orbital periods of particles nearer the planet. An arc ring 15-km wide located 67,000 km from Neptune's centre would spread out to encircle the entire planet in less than 3 yr. Interparticle collisions and, for small particles, Poynting–Robertson drag, would cause radial spreading on a timescale of less than 10^8 yr (ref. 3), which is short compared with the age of the Solar System. Thus, some active confining mechanism is required, unless the arc ring is extremely young.

Torques caused by observed moons are responsible for several sharp boundaries in Saturn's rings^{4–8}. Undetected moonlets are believed to be responsible for confining the uranian rings³, as well as several features in Saturn's rings⁹. Although no discontinuous arc ring is seen (except possibly within the Encke gap in Saturn's A ring⁹), several of the resonantly produced features are eccentric^{4,7,8}. Satellites are thus capable of producing azimuthally variable ring structure.

Jupiter confines the Trojan asteroids to a limited azimuthal range around the stationary triangular (Lagrange) points of the Jupiter–Sun system, L_4 and L_5 . These asteroids are in a 1:1 orbital commensurability with Jupiter, orbiting 60° ahead of and behind the planet. Similar Lagrange point librators share orbits with some of Saturn's many moons^{4,10,11}. One early model of the uranian rings proposed that they were confined in a similar, although less tightly bound, horseshoe resonance lock¹². However, although individual satellites and groups of non-interacting satellites orbit stably in such resonance locks, collisional rings cannot be confined in such a manner. The problem arises because the triangular points are potential maxima (which are only stable because of Coriolis forces¹³), and interparticle

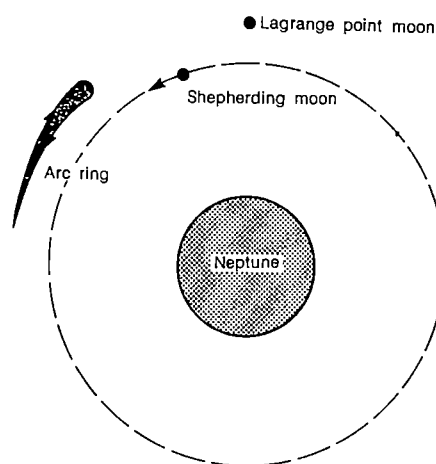


Fig. 1 Neptune's arc ring viewed from above Neptune's pole, as predicted from the present model. The ring appears stationary in the frame rotating with the Lagrange point moon, although the individual ring particles librate slowly about the triangular point in the direct indicated. The frame rotates in the anticlockwise direction with respect to inertial space.

collisions, which dissipate energy, drive particles away from energy maxima. Although a satellite can azimuthally confine ring particles which have semimajor axes (nearly) the same as its own, interparticle collisions and other dissipative effects will cause radial spreading similar to that of an unconfined ringlet.

Saturn's F ring is confined to a radially narrow strand caused by torques from 'shepherding' moons on both sides⁴. Undetected shepherding moons are believed to confine the nine narrow rings of Uranus³. Shepherding is possible because a moon exerts a torque which repels nearby ring material. This torque falls off rapidly with distance from the moon. Ring particles initially orbiting between two moons are thus confined to a ring whose radial width is determined by a balance between diffusive spreading and satellite torques³.

We suggest that the arc ring of Neptune is azimuthally confined near a triangular Lagrange point of an undetected satellite (or, possibly, 1981N1), and radially confined by at least one shepherding moon on a nearby orbit (Fig. 1). One shepherding moon is sufficient because the ring particles' orbits are alternately interior to, and exterior from, that of the confining Lagrange point satellite. The shepherding moon, which may orbit either interior to, or exterior from, the ring, repels all the ring particles. However, when a particle is on the half of its libration cycle which brings it nearer the shepherd, the magnitude of the repulsive torque, which at the time is pushing the ring particle towards the orbit of the Lagrange point moon, is greater than the magnitude of the repulsive torque pushing the particle away from the Lagrange point moon during the other half of its libration cycle. Thus, the shepherd's net effect is to stabilize the 1:1 resonance between the ring particles and the Lagrange point moon. In celestial mechanics terminology, the torque caused by the shepherding satellite acts, on average, to decrease the Jacobi constants of the ring particles, driving them closer to L_4 or L_5 . Note that energy conservation is not violated; the energy lost in inelastic collisions between ring particles is resupplied to the ring from orbital energy of the rings and moons. The shepherding moon is repelled from the ring in the process, but the timescale for this to occur may be longer than the age of the Solar System. (Alternatively, tidal torques on the moons by Neptune may counteract this repulsion.)

The combination of a Lagrange point moon and a shepherding moon on a nearby orbit is thus qualitatively capable of producing the arc ring structure observed around Neptune. Quantitative estimates will now be made which show that Neptune's ring may be confined by moons of sufficiently small size that they would not have been photographically detected from Earth.

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To zero order, the orbits of the ring particles are keplerian ellipses about Neptune. The semimajor axes of these ellipses are perturbed by the lagrangian moon, causing the ring particles to librate about one of the moon's triangular points with periods of the order of 1 yr. Perturbations by the shepherding moon induce eccentricities in the ring particles. The damping of these eccentricities results in a radial drift on a much longer timescale, comparable with the timescale for diffusive spreading of the ring.

The relationship between the radial, δr , and angular, θ , separations of a given ring particle from the Lagrange point satellite is:

$$\frac{3}{4} \left(\frac{\delta r}{r} \right)^2 + \frac{m}{M} \left(\frac{1}{\sin(\theta/2)} - 2 \cos \theta + 2 \right) = C - 3 \quad (1)$$

where r is the satellite's semimajor axis, m is the mass of the Lagrange point moon, M is Neptune's mass, and C is the Jacobi constant¹⁴. The Jacobi constant depends on the amplitude of the particle's libration about the triangular point, and differs for different ring particles. For a given ring particle, the Jacobi 'constant' varies slowly, in response to torques from the shepherding moon and interparticle collisions. The width of a particle's libration at a given angle can be computed from equation (1) to be:

$$w = 2\delta r = \frac{4r}{\sqrt{3}} \left\{ C - 3 - \frac{m}{M} \left(\frac{1}{\sin(\theta/2)} - 2 \cos \theta + 2 \right) \right\}^{1/2} \quad (2)$$

and the length of the arc, l , is:

$$l = r(\theta_2 - \theta_1) \quad (3)$$

where the θ_i are the angles at which $\delta r = 0$ (see equation (1)). Presumably, the shepherding moon confines the ring particles to a maximum value of C (and thus of δr). If the arc ring subtends no more than 10% of the circumference of the satellites orbit, and its maximum width is at least 15 km, then a lower bound on the lagrangian satellite's mass is 4.5×10^{21} g; this bound may be reduced to 0.48×10^{21} g if an arc of 156° (the maximum possible libration amplitude for tadpole orbits) is allowed. Assuming a density of 1 g cm^{-3} , a moon of mass 4.5×10^{21} g would have a diameter ~ 200 km. The tentatively detected moon 1981N1, which presumably has a diameter between 100 and 1,000 km (ref. 2), is thus a candidate for the Lagrange point satellite.

The size(s) of the shepherding satellite(s) are less well constrained. The torque per unit ring mass, T_s , on a ring at radius r caused by a shepherding satellite of mass m_s at radius r_s is^{3,15}:

$$T_s = 0.4 \text{ sign}(r - r_s) \left\{ \frac{Gm_s}{\Omega(r - r_s)^2} \right\}^2 \quad (4)$$

where Ω is the angular frequency of the ring particles. The differential shepherding torque across the ring must balance the viscous and other diffusive torques within the ring (including the extra diffusive torque caused by stirring by the shepherding moon¹⁶). Assuming just one shepherding moon, its mass would be roughly:

$$m_s \sim 3 \times 10^{21} \left(\frac{\nu}{10} \right)^{1/2} \left(\frac{r - r_s}{10^8} \right)^{5/2} \quad (5)$$

where ν is the effective kinematic viscosity of the ring and all quantities are in c.g.s. units. Estimates of ν for Saturn's rings range between 5 and $260 \text{ cm}^2 \text{ s}^{-1}$ (refs 8, 17). Thus, the shepherding moon would be ~ 120 km in diameter if its orbit was 1,000 km from that of the ring; the required diameter would scale slightly slower than linearly with its distance from the ring. An arc ring which has a maximum width $\gg 15$ km would permit a smaller shepherd for a given separation and viscosity; however, it would require a significantly larger Lagrange point satellite. If there were shepherds on both sides of the ring, required masses would decrease by a factor of $\sqrt{2}$.

Thus, Neptune's arc ring may be confined by two nearby moons each ~ 100 – 200 km in diameter. One of the moons would

restrain the ring azimuthally near one of its stable triangular points with Neptune. The other moon, circulating on a different orbit, would confine the ring radially in the standard shepherding manner. Other configurations are also possible. For example, the shepherding moon may be coorbital with the Lagrange point moon, but have a larger libration amplitude than that of the ring. The problem with this latter scenario is that the torque from the ring would drive the shepherd away from the coorbital resonance; either the moons would have to be much more massive than the ring (so the timescale would be very long) or the system would have to be supplied with energy from an external source (such as tidal torque from the planet).

Further observations of Neptune's arc ring by stellar occultations, the Hubble Space Telescope and, in 1989, by the Voyager 2 spacecraft, should greatly increase our knowledge of this newly discovered structure, and determine whether the model presented here is accurate. A search for arc rings orbiting the other giant planets may also prove fruitful.

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Timescale-invariant profile of the type II bursts from the Rapid Burster

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The Rapid Burster is a unique X-ray source which produces rapidly repetitive X-ray bursts. Each rapid burst shows a characteristic structure in its decay part comprising successive peaks. We have now discovered that the structure of each individual burst shows a nearly identical profile except for the timescale, which differs largely from burst to burst; the profile of the decay is timescale invariant. This invariance holds over a wide range of timescales to at least a factor of 30. Furthermore, we have found that the heights of successive peaks as well as the intervals between them decrease with time according to a common arithmetic rule.

The rapidly repetitive bursts from the Rapid Burster are probably due to chopped accretion flow caused by some instabilities¹. Hoffman *et al.*² designated these bursts type II bursts and those interpreted as thermonuclear flashes as type I bursts. Here, we shall discuss the remarkable characteristics of the structure in the decay part of type II bursts from the Rapid Burster.

We observed the rapid burst activities of this source from Tenma in August 1983 (ref. 3) and July 1984. Each burst exhibits

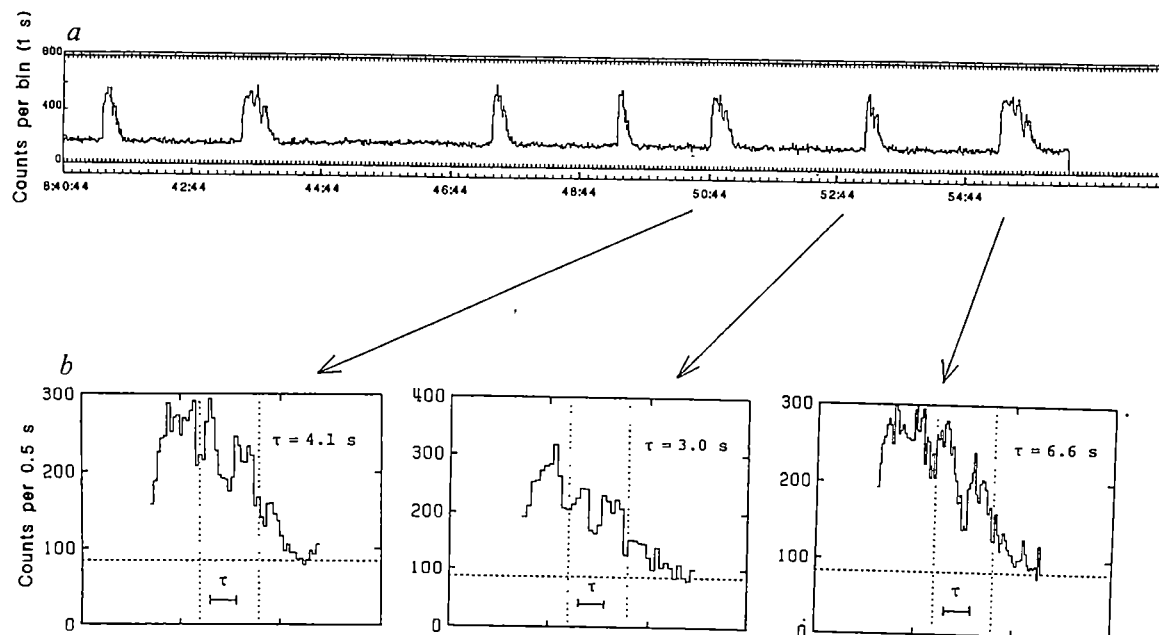


Fig. 1 *a*, A portion of the rapid burst activity (1983). *b*, Detail of the profiles of three bursts with different length. Timescale in these diagrams is in units of the characteristic time τ which is determined from a cross-correlation analysis performed between the two dashed lines for each burst.

a significant structure in the decay part. Long flat-topped bursts with durations exceeding 40 s show one or two humps in the decay. Bursts of shorter duration commonly comprise more than two peaks (Fig. 1). Clearly, the timescale of the structure depends on the burst duration; the longer the burst duration, the slower is the excursion of the decay structure.

There is a striking similarity in the form of the decay structure between bursts, except that the timescale of the structure differs from burst to burst. In order to examine the similarity in more detail, we performed a cross-correlation analysis of the decay profile between bursts by varying timescales, and determined the relative timescale for each burst. Cross-correlation was taken between the dashed lines shown in Fig. 1. For convenience, we define the characteristic time τ for each burst as being the interval between the first and second peaks in the decay, as also indicated in Fig. 1.

We then constructed composite profiles from many bursts by normalizing the characteristic times, as shown in Fig. 2 for different ranges of τ . The number of bursts superimposed is indicated on each figure.

In Figs 1 and 2 we see that the decay structure is essentially independent of the timescale. In other words, the profile of the decay is timescale invariant and can be expressed by a single function $F(t/\tau)$ with time t . Obviously, the profile is not of a simple damping oscillation but more complex. For instance, the second and the third peaks are close together and form a broad hump. Such fine detail is not eliminated by superimposing many bursts. This fact implies that, independently of the length of timescale of each burst, the structure is intrinsic and solid, as in the case of the X-ray pulsar profiles. Furthermore, we stress that the timescale invariance of the decay profile holds for a wide range of the characteristic time τ from 9 to 0.3 s, the shortest limit with our time resolution.

As regards the detail of the decay structure, we note that there is a specific trend in the heights and intervals between successive peaks. First, the peak height appears to decrease linearly with time. Second, the interval is uneven and gradually shortened towards the end of decay. The observed pattern indeed suggests the presence of a certain regularity.

We discovered an interesting rule that satisfactorily determines the relative positions of the successive peaks. Suppose we assign the positions of the broad main peak (0th peak) and the following peaks, respectively, as indicated in Fig. 3. We find

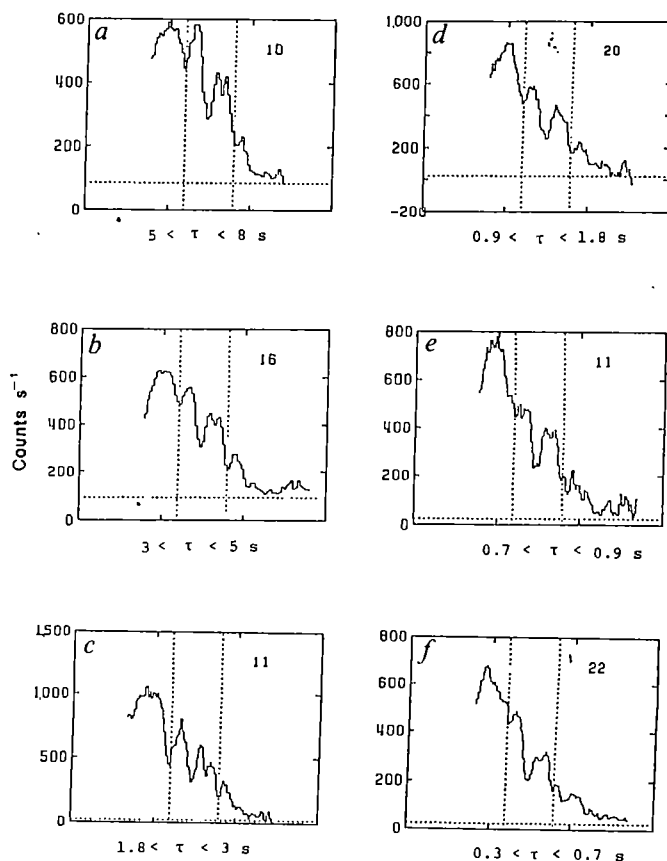


Fig. 2 Composite profiles of type II bursts constructed by normalizing the characteristic times, for six different ranges of τ . The number of bursts superimposed is indicated in each panel. *a-c* are from 1983 August data taken with a time resolution of 0.5 s, and *d-f* are from 1984 July data with a time resolution of 0.125 s.

that $(X_4 - X_2)/(X_2 - X_0) = (X_5 - X_3)/(X_3 - X_1) = \alpha$, with α approximately 0.57, where X_n is the time of the n th peak in units of the characteristic time. In other words, the successive peak positions are 'phased' in such a way that the intervals between the even-numbered peaks and those between the odd-

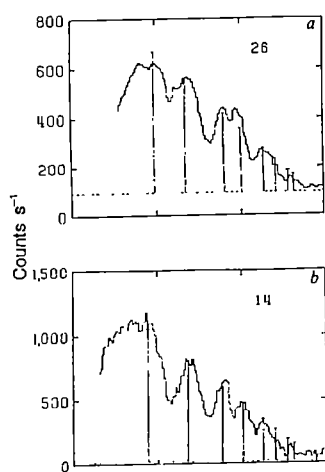


Fig. 3 Composite profile of the type II bursts of 1983 ($2.5 < \tau < 3.5$ s) (a) and 1984 ($2.0 < \tau < 3.5$ s) (b) activities. Vertical bars indicate the positions and heights of the peaks determined as described in the text.

numbered peaks respectively compose a geometric series with the same multiple α , although the peaks after the fourth are not always statistically significant. From the relative phase of the odd-numbered peaks with respect to the even-numbered ones, we define $(X_3 - X_1)/(X_2 - X_0) = \beta$, then $(X_1 - X_0)/(X_2 - X_0) = (1 - \beta)/(1 - \alpha)$, so that the geometric series of the even-numbered and odd-numbered peaks converge at the same point.

The same rule seems to hold for the heights of successive peaks. Namely, the successive peak heights, Y_n for the n th peak, roughly satisfy the following relations: $Y_2/Y_0 = Y_4/Y_2 = Y_6/Y_4 = \alpha$ for the even-numbered and the odd-numbered peaks, with the same value of α as determined for the respective intervals; besides, $Y_1/Y_0 = \beta$. Thus, the common rule that applies to the intervals and heights makes the successive peaks appear to decrease linearly with time.

The above rule holds equally for the rapid bursts observed in 1983 as in 1984. There is a significant difference in the peak luminosity as well as the time-averaged luminosity between the activities of these two years. The bursts in the 1984 activity are on average roughly twice as luminous as those of 1983. The time-averaged luminosity during the 1984 activity is also about double that of 1983. It is important that, despite this difference, the values of α are the same in the two separate activities. However, a subtle difference exists in the relative phase of the odd-numbered peaks with respect to the even-numbered peaks. In the 1983 bursts, the odd-numbered peaks are shifted slightly earlier in phase than the corresponding peaks in the 1984 bursts; this is most noticeable in the smaller separation between the second and third peaks of the 1983 profile. The difference is expressed in terms of β which are 0.81 and 0.77 for the activities in 1983 and 1984, respectively.

If one assumes that the luminosity of a burst at a given time is determined by the instantaneous accretion rate, the above findings on the decay structure imply the presence of some complex flow-control mechanism. One might speculate that the structures are already prepared in the accretion disk (for example, regular concentric rings similar to the Saturn's rings). However, any model should explain the facts that the timescale-invariant profile is reproduced over a very wide range of burst duration while the peak luminosity (peak accretion rate) is independent of the burst duration and that the pattern is not influenced by the change of average accretion rate.

Search for a heavy neutrino in the β -decay of ^{35}S

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Recent experimental evidence¹ suggests that the neutrino (strictly, an antineutrino) emitted in the β -decay of tritium is a mixture of two mass eigen states $|\nu_1\rangle$ and $|\nu_2\rangle$ where $|\nu_e\rangle = \cos \theta |\nu_1\rangle + \sin \theta |\nu_2\rangle$ with $m_{\nu_1} < 50$ eV and $m_{\nu_2} = 17.1 \pm 0.2$ keV and $\sin^2 \theta = 0.03$. This conclusion is based on the observation of a 'kink' in the Fermi-Kurie plot^{2,3} of the electron energy spectrum at an energy 17.1 keV below the end point. Such an observation, if confirmed, will have far-reaching consequences in cosmology and particle physics^{4,5}. In the experiment reported here, a search was made for such an admixture in the β -decay of ^{35}S . We fail to confirm the presence of mixing of neutrinos with mass 17.1 keV and place an upper limit on such an admixture of 0.6% (90% confidence level).

The β -decay of ^{35}S has been studied extensively in magnetic spectrometers^{6,7}. ^{35}S decays with a half-life of 87.4 days to the ground state of $^{35}\text{Cl}(3/2^+ \rightarrow 3/2^+)$ with 100% branching with an end-point energy of 167.4 ± 0.2 keV (ref. 8). The transition is an allowed one, with a $\log ft = 5.0$. The linearity of the Fermi-Kurie plot has been established down to an energy of 20 keV (ref. 7). However, from the earlier data, particularly from the Fermi-Kurie plots which are usually available in the literature, it is not easy to discover any discrepancy like a 'kink' at around 150 keV, which is expected if a heavy neutrino admixture is present.

In the present experiment, a ^{35}S source in the form of BaSO_4 of less than $10 \mu\text{g cm}^{-2}$ thickness, was deposited onto a $90 \mu\text{g cm}^{-2}$ aluminized polypropylene foil. The β spectrum was measured with a shielded windowless liquid nitrogen-cooled Si(Li) detector of diameter 28 mm and thickness 3 mm fabricated in our laboratory. The detector had a resolution of 3.5 keV at 120 keV. The spectrometer was calibrated with conversion electron lines from ^{57}Co and $^{99\text{m}}\text{Tc}$ and γ -ray lines from $^{114\text{m}}\text{In}$ and ^{137}Cs . The conversion electron spectra were used to obtain the response function of the detector. The back-scattered fraction of electrons from the Si(Li) detector, in the energy range of

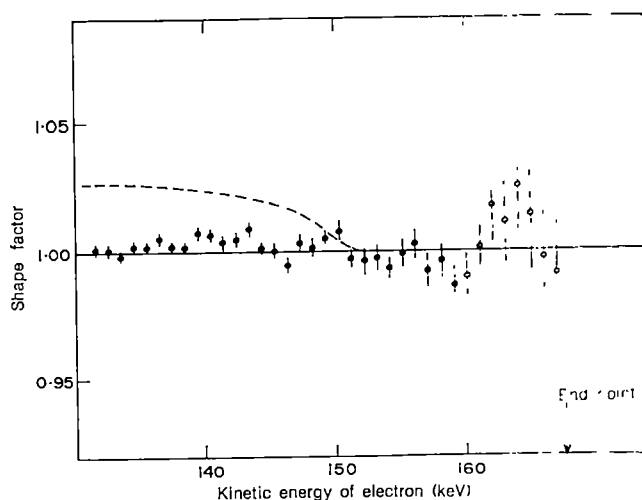


Fig. 1 The shape factor of the β -spectrum of ^{35}S as obtained in the present experiment (\bullet). Error bars, ± 1 s.d. Also shown (---) is the expected shape for a 3% branch in this β -decay where a 17-keV neutrino is emitted, with $\sin^2 \theta = 0.03$.

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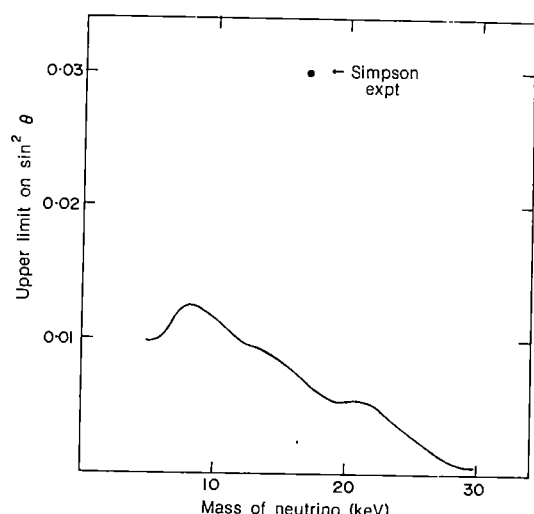


Fig. 2 Upper limit on $\sin^2 \theta$ at the 90% confidence level, obtained from the present experiment and shown as a function of the assumed neutrino mass. The region above the curve is excluded by this experiment. The single point shown at the top represents the result derived from Simpson's experiment¹.

130 keV, was determined to be 0.20 ± 0.02 at a source-detector distance of 8 cm, as used in the experiment. From a comparison of the electron and γ spectra of ^{99m}Tc , a dead layer of the order of 2 mg cm^{-2} was found to be present on the entrance window of the detector. Such a dead layer would not allow an accurate measurement of the β spectrum down to very low energies of $\sim 50 \text{ keV}$. However, this would not appreciably affect the shape of the spectra in the limited energy range 130–170 keV, hence we restricted our analysis to this energy region after correcting for the energy loss in the dead layer.

The counting rate was kept below 1,000 Hz and a time constant of $0.5 \mu\text{s}$ was used for the amplifier. Perspex collimators were used to block the electrons scattered from the chamber walls reaching the detector. These collimators also reduced the external bremsstrahlung produced from the walls of the vacuum chamber. The efficiency of the detector was determined from 75 keV to $\sim 1 \text{ MeV}$ using the internal conversion lines of the decay of ^{152}Eu . The measured efficiency was found to be constant to within $\pm 3\%$ in this energy region. By interpolation, the energy variation of efficiency can be estimated to be less than $\pm 0.2\%$ over the small energy range (130–170 keV) in which the data have been analysed. The measured β -spectrum was corrected for background and pile-up effects. The theoretical β spectrum for ^{35}S was calculated for $m_\nu = 0$ using the Fermi functions of Behrens and Janecke⁹ and was folded with detector response function. The ratio of the experimental to the calculated spectrum normalized to 1 is the shape factor, and is shown in Fig. 1. The measured end point obtained is in agreement with the accepted value. If there is an admixture of heavy neutrinos of mass m with a mixing amplitude $\sin \theta$, such a shape factor $C(W)$ would have the form

$$C(W) = \cos^2 \theta + \sin^2 \theta \left[1 - \frac{m_\nu^2}{(W_0 - W)^2} \right]^{1/2} \text{ for } W < (W_0 - m_\nu)$$

$$C(W) = \cos^2 \theta \text{ for } (W_0 - m_\nu) < W < W_0$$

where W is the electron energy and W_0 the end-point energy. Also shown in Fig. 1 (broken curve) is the expected shape factor, with assumed values of $\sin^2 \theta = 0.03$ and $m_\nu = 17 \text{ keV}$ as reported by Simpson¹. It is clear from the figure that such a curve lies well outside our experimental limits, ruling out the existence of a mixture of a heavy neutrino of mass 17 keV and $\sin^2 \theta = 0.03$, in the β -decay of ^{35}S . In Fig. 2 the upper limits for $\sin^2 \theta$, at the 90% confidence level, derived from the above shape factor are shown as a function of the mass of the neutrino. It is clear that a heavy neutrino admixture of more than 1% does not exist in ^{35}S β -decay for the neutrino mass range 5–30 keV. Our results,

together with those of Schreckenbach *et al.*,¹⁰ do not suggest the presence of a heavy neutrino admixture above 1% for the neutrino mass range of 5–460 keV.

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Solid solution in plumbous potassium oxysilicate affected by interaction of a lone pair with bond pairs

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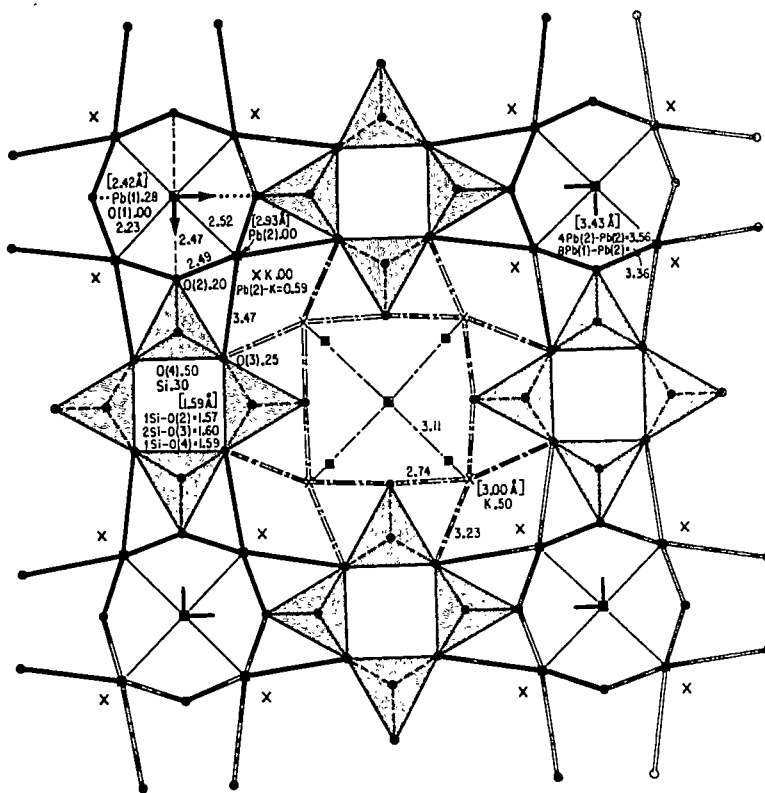
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During explorations of the chemical crystallography of lead silicates, the synthetic phase $\text{K}_2\text{Pb}_4\text{Si}_8\text{O}_{21}$ (here KPS), attracted our attention. Clear crystals when ground under acetone produced fine needles and fluffy aggregates which resembled chrysotile asbestos and had elicited some earlier interest¹. Evidence indicated tetragonal symmetry and despite several attempts no report of a successful structure solution has appeared. Indeed, the structure analysis proved to be a challenge, and revealed $^{1/2}[\text{Si}_8\text{O}_{20}]$ tubular columns and $[\text{Pb}_2\text{OPb}_4\text{O}_{24}]$ clusters, the latter consisting of six Pb^{2+} cations situated on the vertices of a compressed octahedron. A novel feature is the occurrence of disordered (Pb^{2+} , K^+) as a 'solid solution', the $\text{Pb}-\text{K} = 0.59 \text{ \AA}$ separation being a consequence of the $6s^2 \text{ Pb}^{2+}$ lone pair acting on circumjacent bond pairs. In our initial experience, best convergences yielded the reliability index $R \sim 0.20$. Unit cell parameters, space group and other structure cell criteria in Table 1 show good agreement between the present study and an earlier report² on KPS and isostructural $\text{Pb}_2\text{Pb}_4\text{Si}_6\text{Al}_2\text{O}_{21}$.

Our data set, on a crystal which was initially checked with assorted precession photographs and yielded condensed X-ray symbol $\text{I4}/***$, included 421 unique reflections for refinement. The data set was collected with an Enraf Nonius CAD-4 automated diffractometer utilizing graphite monochromator and $\text{MoK}\alpha_1$ radiation. All 3,326 reflections for $2\theta \leq 60^\circ$ were collected, and each was corrected for absorption based on an empirical ψ -scan, and symmetry-equivalent reflections were averaged. Unit cell dimensions (Table 1) were obtained by least-squares refinement of 25 reflections distributed throughout reciprocal space.

The final crystal structure was deciphered quite serendipitously. Three-dimensional Patterson maps quickly revealed the Pb-Pb vectors, and attempts were made to locate the remaining Pb-X (X = K, Si, O) vectors through models constructed in space group $\text{I4}/mmm$; and its subgroups I4mm , I42m , I4m2 and I422 . A $\text{Pb}(1)$, $\text{Pb}(2)$ and K combination was introduced to a least-squares refinement, but the $\text{Pb}(2)$ -K site distance was

Fig. 1 Structure diagram of $\text{Pb}_2\text{O}(\text{Pb}_2\text{K}_2)[\text{Si}_8\text{O}_{20}]$ down $[001]$. The silicate tubes appear as stippled tetrahedra. Oxygens are located at solid disks, Pb atoms at squares and K atoms at crosses. Note O(1) superimposes on Pb(1) in this projection. Pb(2)–O bonds (solid) and Pb(1)–O bonds (dashed) are shown around the origin of the cell, K–O bonds (dot-dash) around body centre at $(\frac{1}{2}, \frac{1}{2}, \frac{1}{2})$. Individual bond distances (in Å, errors < 0.01 Å) and polyhedral averages (in brackets) are shown adjacent to their geometric elements. Heights of atoms along c are given in fractional coordinates for their asymmetric unit.



only 0.6 Å. Surprisingly, $R = 0.10$. It then became clear that an apparent solid solution existed between Pb(2) and K. From that model, all other atoms could be located on a Fourier synthesis, which was based on space group $I4/mmm$. Continued coordinate parameter refinement followed by full-matrix anisotropic thermal vibration parameter refinement led to $R = 0.034$. Atom coordinate parameters from the final refinement appear in Table 2. We are forced to conclude that Pb(2) and K are coupled, both Pb(2) and K sites each half-populated about a centroid at $(\frac{1}{2}, \frac{1}{2}, 0)$. As we discuss below, these results are a consequence of an effect on solid solution from the interaction of $6s^2$ Pb $^{2+}$ lone pair with neighbouring bond pairs. The compound is a plumbous potassium oxysilicate, more specifically $\text{Pb}_2\text{O}(\text{Pb}_2\text{K}_2)[\text{Si}_8\text{O}_{20}]$.

KPS is structurally related to some other well-defined arrangements. Figure 1 shows K–O, Pb–O bonds and $[\text{Si}_8\text{O}_{20}]$ polyhedral linkages; Bond distances and polyhedral averages are also presented. Two distinct regions or fundamental building blocks can be discerned which, when isolated, show a direct relationship to some other structure. The first region is the $[\text{Si}_8\text{O}_{20}]$ column or tube which runs parallel to the c -axial direction. Perhaps the simplest way to describe this silicate tube is by corner-linked condensation of $[\text{Si}_2\text{O}_7]$ sorosilicate units, the tube generated by the 4_2 -screw operation parallel to the c -axis implicit in space group $I4/mmm$. This motif occurs in several distinct structure types, the simplest³ being narsarsukite, $\text{Na}_4\text{Ti}_2\text{O}_2[\text{Si}_8\text{O}_{20}]$. In the structure we report, the tube is more rigid, constrained by the presence of additional mirror planes which are normal to the a_1 and a_2 axial directions. The second region was an unexpected surprise. It is centred on O(1) at the

origin of the unit cell, and has local stoichiometry $\text{Pb}(1)_2\text{O}(\text{Pb}(2)_4\text{O}_{24})$, selecting the Pb end-member. A $\text{Pb}(1)_2\text{Pb}(2)_4$ cation cluster surrounds O(1), and is a compressed octahedron, with eight apical Pb(1)–Pb(2) = 3.36 Å and four equatorial Pb(2)–Pb(2) = 3.56 Å, with an average of Pb–Pb = 3.43 Å. From the individual polyhedral bond distances, it is evident that the $6s^2$ lone electron pairs in Pb $^{2+}$ are situated on the outside of the $[\text{Pb}_6]$ octahedron in KPS. Note that the interatomic separation⁴ in Pb metal (face-centred cubic, f.c.c.) is 3.500 Å. This does not imply metallic Pb–Pb bonds in KPS, since the intervening anions and Pb–O bonds in the silicate must be included.

The Pb(2) and K sites, their half-occupancy the source of difficulty in structure solution, have separation K–Pb(2) = 0.59 Å. Decomposing the cluster into its polyhedral components, the $\text{Pb}(1)\text{O}(1)\text{O}(2)_4$ or $\text{Pb}(1)\text{O}_5$ defines a square pyramid of oxygen atoms coordinated to Pb(1). Alternatively, it can be described as an octahedron about Pb(1) with one vertex representing the lone electron pair (symbolized ψ) from $6s^2$ Pb(1) $^{2+}$. The Pb(1) atom is displaced 0.59 Å away from the equator of the $\text{Pb}(1)\text{O}_5\psi$ octahedron and towards the direction of the lone pair. Similar coordination polyhedra have been reported in several structures, although refinements which involve at least one heavy metal in an oxide matrix were usually inferior. A good example is the $\text{PbO}_4\psi$ polyhedron in the structure of tetragonal PbO or litharge, and refined from neutron diffraction data⁵. The shortest distance, Pb(1)–O(1) = $2.228(1)$ Å, opposes the assumed centroid of the lone pair. In litharge, the Pb atom is displaced 1.19 Å away from the equator and towards the direction of the lone pair. The average Pb–O distance in PbO_4 for litharge, a square pyramid with Pb as the apex, is 2.31 Å, significantly shorter than the 2.42 Å average for KPS. The short basal O–O = 2.80 Å separation in litharge contrasts with the tetragonal base distance O(2)–O(2) = 3.26 Å in KPS. These shorter distances in litharge are probably based on differences in oxygen coordination number about Pb(1), being 4 for litharge and 5 for KPS.

A large family of structures involves components Pb–X–O–H–Cl where X = Cu $^{2+}$, Mn $^{2+}$, Bi $^{3+}$, Sb $^{3+}$, As $^{3+}$, and so on, and many are based on similar structural principles. One well-refined structure⁶ includes diabolite, $\text{Pb}_2\text{Cu}(\text{OH})_4\text{Cl}_2$, with tetragonal space group $P4mm$ and one formula unit in the structure cell.

Table 1 Cell-structural data for $\text{Pb}_2\text{O}(\text{Pb}_2\text{K}_2)[\text{Si}_8\text{O}_{20}]$

	This study	Gibbs <i>et al.</i> ²	Narsarsukite ³
a (Å)	11.806(2)	11.816(6)	10.726(1)
c (Å)	7.913(2)	7.932(4)	7.947(1)
Z	2	2	2
Space group	$I4/mmm$	$I4/mmm$	$I4/m$
Specific gravity		4.40	2.78(1)
Density (g cm $^{-3}$)	4.418		2.776
Formula	$\text{Pb}_2\text{O}(\text{Pb}_2\text{K}_2)[\text{Si}_8\text{O}_{20}]$		$\text{Na}_4\text{Ti}_2\text{O}_2[\text{Si}_8\text{O}_{20}]$

Table 2 $\text{Pb}_2\text{O}(\text{Pb}_2\text{K}_2)[\text{Si}_8\text{O}_{20}]$: atom coordinates and anisotropic thermal vibration ($\times 10^4$) parameters*

Atom	Cell	x	y	z	U_{11}	U_{22}	U_{33}	U_{23}	U_{13}	U_{12}
Pb(1)	4	0.0000	0.0000	0.2815(1)	405(4)	405(4)	93(3)	0	0	0
Pb(2)	$8 \times \frac{1}{2}$	0.1508(1)	0.1508(1)	0.0000	116(3)	116(3)	229(5)	0	0	-32(5)
K	$8 \times \frac{1}{2}$	0.1860(5)	0.1860(5)	0.0000	210(23)	210(23)	165(29)	0	0	57(30)
Si	16	0.3197(2)	0.0000	0.3006(3)	99(11)	298(14)	121(10)	0	-14(9)	0
O(1)	2	0.0000	0.0000	0.0000	645(119)	645(119)	148(106)	0	0	0
O(2)	16	0.2028(5)	0.0000	0.2057(7)	134(29)	277(34)	224(31)	0	-61(27)	0
O(3)	16	0.1113(5)	-0.3887(5)	0.2500	292(25)	292(25)	1,095(78)	-42(37)	42(37)	185(36)
O(4)	8	0.3031(12)	0.0000	0.5000	287(75)	2,970(273)	106(51)	0	0	0

The polyhedron of interest is $[\text{Pb}^{2+}\text{O}_4\text{Cl}_4]$ which is a distorted square antiprism. There exist $4\text{Pb}-\text{OH}=2.48$ and $4\text{Pb}-\text{Cl}=3.32$ Å averages. Electing $r=1.40$ Å for $^{[6]}\text{O}^{2-}$, $r=1.81$ Å for $^{[6]}\text{Cl}^-$ and $r=1.29$ Å for $^{[8]}\text{Pb}^{2+}$, the calculated averages are $\text{Pb}^{2+}-\text{O}=2.69$ Å and $\text{Pb}^{2+}-\text{Cl}=3.10$ Å, effective averages larger and smaller, respectively, than the experimental averages for diabolite. This was noted in the earlier study which further supports the interaction between the $6s^2$ Pb^{2+} lone pair with the enveloping bond pairs. It requires that the centroid of the lone electron pair is towards the direction of the plane of the Cl^- ions, foreshortening the opposing $\text{Pb}^{2+}-\text{OH}^-$ distances and lengthening the adjacent $\text{Pb}^{2+}-\text{Cl}^-$ distances with respect to the effective averages. An analogous relation can be found for $\text{Pb}(1)\text{O}_5\psi$ in KPS where $4\text{Pb}(1)-\text{O}(2)=2.47$ Å and $1\text{Pb}(1)-\text{O}(1)=2.23$ Å. If the centroid of the lone pair is postulated to oppose O(1) in KPS; then the short $\text{Pb}(1)-\text{O}(1)$ distance and the absence of $\text{Pb}^{2+}-\text{O}$ bonds on its opposing side can be understood. It is immediately apparent that the average for the Pb ion stripped of all its valence electrons, $^{[6]}\text{Pb}^{4+}-\text{O}=2.18$ Å from the same tables⁷, is not far removed from $\text{Pb}(1)-\text{O}(1)$ in KPS. Increase in average distances for coordination polyhedra of increasing order has been explained adequately elsewhere⁸. Similarly, displacement of a metal with a lone electron pair from the equator of a coordination polyhedron has electrostatic origins in the interaction implicit on a cation with a lone pair of electrons and the circumjacent bond pairs⁹.

The remaining polyhedron in the second region is a distorted monocapped cube, or $\text{Pb}(2)\text{O}_9$. The average distance is $\text{Pb}(2)-\text{O}=2.93$ Å, but the range in polyhedral distances is considerable, from $\text{Pb}(2)-\text{O}(2)=2.49$ Å to $\text{Pb}(2)-\text{O}(3)=3.47$ Å. We propose that the locus of the lone pair is on $\text{Pb}(2)$ opposite the $\text{Pb}(2)-\text{O}(1)$ bond, the considerable distortion in polyhedron and bond distances being due to the effect from interaction of a lone pair with the surrounding bond pairs. Further evidence for this interaction can be obtained from the same structure by selecting the site centroid of the KO_9 polyhedron associated with the purported $[\text{K}, \text{Pb}(2)]$ solid solution. The distances range from $\text{K}-\text{O}(2)=2.74$ Å to $\text{K}-\text{O}(3)=3.23$ Å with an average $\text{K}-\text{O}=3.00$ Å, a range of only 0.49 Å compared with a range of 0.98 Å for $\text{Pb}(2)-\text{O}$. This is a rare case where a cation with a lone pair of electrons in the valence shell can be directly compared with a cation where all valence electrons are stripped away in the same polyhedral environment which, in effect, constitutes a structural solid solution (compare with isostructural $\text{Pb}_2\text{Pb}_4\text{Si}_6\text{Al}_2\text{O}_{21}$).

Another feature is the similarity of the cluster in the second region to the structure of hyalotekite, a complex lead-barium-calcium-borosilicate-fluoride¹⁰. This compound is triclinic, but its cell is pseudo-tetragonal, with $a \sim 11.1$ Å, $c \sim 10.3$ Å and possesses two formula units in the cell. A weak structural relationship occurs between KPS and hyalotekite. Writing the formulae in sequence hints at this relationship.

KPS $\text{Pb}_2\text{O}(\text{Pb}_2\text{K}_2)$ $[\text{Si}_8\text{O}_{20}]$ $\text{Pb}-\text{K}=0.59$ Å
Hyalotekite $\text{Ca}_2\text{F}(\text{Pb}_2\text{Ba}_2)$ $[\text{Be}_{0.5}\text{B}_2\text{Si}_{9.5}\text{O}_{28}]$ $\text{Pb}-\text{Ba}=0.46$ Å

In both structures, an apparent solid solution exists between a cation possessing a lone pair and a cation with a closed shell.

The KPS structure invites a simple experiment. The effect on a solid solution from interaction of a lone pair and neighbouring bond pairs with respect to temperature is unknown. Even the problem of solid solution between a cation possessing a lone pair (such as Pb^{2+}) and a cation stripped of all its valence electrons (such as K^{1+}) is not understood. We plan to study the crystal structure of KPS at elevated subsolidus temperatures to explore in more detail the bond distances and behaviour of the anisotropic thermal vibration ellipsoids through the thermal effect on the $\text{Pb}(2)-\text{K}$ site separation. Finally, since the lone electron pairs about Pb^{2+} are evidently circumjacent to the second aforementioned structural region and since the first region is a silicate tube, the curious appearance of fluffy fibrous material on grinding clear crystals suggests a pressure effect on the interaction between a lone pair and circumjacent bond pairs. Bond length, bond angle and solid solution dependencies on temperature and pressure would be most informative.

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An interhemispheric comparison of the concentrations of bromine compounds in the atmosphere

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At least seven organic bromine compounds have been positively identified in the atmosphere^{1,2} (CH_3Br , CH_2Br_2 , CHBr_3 , CH_2BrCl , CF_3Br , CF_2BrCl and $\text{C}_2\text{H}_4\text{Br}_2$) and others have been observed in coastal seawater samples ($\text{C}_2\text{H}_5\text{Br}$, $\text{C}_3\text{H}_7\text{Br}$ and CHBr_2Cl) (S. A. P. and R. A. Rasmussen, unpublished data). The atmospheric chemistry of bromine compounds has considerable consequence in the stratosphere, where bromine acts as a more efficient catalyst than chlorine in removing ozone^{3,4}. Several

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bromine compounds also fulfil an important role in the geochemical cycling of the element through the troposphere⁵. Here we present measurements of four bromine compounds (CF_3Br , CH_3Br , CH_2Br_2 and CHBr_3) over a large latitudinal range (40°N to 75°S). These data suggest that the major source of bromine in the atmosphere could be bromoform (CHBr_3), probably emitted from the ocean and with a short lifetime due to photolysis. Our data also suggest that the major emission of methyl bromide (CH_3Br), and hence most bromine entering the stratosphere, will be anthropogenic.

The specific sources of many bromine compounds are unknown, but are either anthropogenic or natural, with ocean chemistry and volcanic activity being obvious choices for the latter. The atmospheric lifetimes of the individual molecular species are likely to differ by many orders of magnitude, depending on the reaction rate with hydroxyl radicals or the photolytic destruction rate.

One way to categorize the source type and reactivity is to collect information on the latitudinal and longitudinal variation of the concentration of these compounds, in particular, to determine whether there is a substantial difference in concentration between Northern and Southern Hemispheric air samples. This is because the vast majority of anthropogenic sources are situated in the Northern Hemisphere and the interhemispheric transfer time is quite long (1–4 yr)^{6,7}.

Measurements of this type have been made at Harwell on air samples collected by the British Antarctic Survey (BAS) whose ships regularly traverse the Atlantic and Southern Oceans from about 50°N to 75°S on route from the UK to Antarctica. The samples were collected using a cryogenic trapping technique originally devised by Rasmussen⁸. Air was sampled on the bow of the BAS ships *RRS Bransfield* through a 10-ft length of steel pipe, which protruded out from the plating, and the sampling time was typically 10 min. The samples were only collected when the wind was on the bow or from a non-contaminating quarter, so the potential for contamination from the ship was very small. Typically 50 l of air were collected in each of the stainless steel sample bottles (volume 1.6 l), which were made to high specifications by Rasmussen. The properties of these containers with respect to contamination and decay of many trace gases have been studied extensively. Almost all trace gases were found to be stable at the high pressures (~ 450 p.s.i.) resulting from the sample collection process, when the air contained amounts of water vapour typical of surface samples. The wind direction was recorded at the time of sampling. No air mass trajectory calculations have been made due to difficulty in obtaining meteorological data.

In 1982, the ship sailed from Southampton to Cape Town, South Africa, passing the Canary and Ascension Islands along standard steamship routes and then to Antarctica. In 1983, the ship sailed from Southampton to Rio, Brazil, passing the Canary Islands and about 100 km east of the Cape Verde Islands along the steamship route and then on to Antarctica. The samples collected as far as Cape Town and Rio were air-freighted to London and analysed at Harwell within 6 weeks of collection. The samples collected farther south arrived back in England up to 6 months after collection. No marked difference in trace gas concentrations could be observed due to the different storage times.

The samples were analysed at Harwell for a large range of trace gases including halocarbons, hydrocarbons and sulphur compounds. In the case of the bromine compounds, which were the subject of this investigation, analysis was made using a highly sensitive gas chromatograph/mass spectrometer (GC/MS) combination developed especially for atmospheric trace gas measurements⁹. Before injection into the GC the air sample was enriched using a procedure very similar to that described previously¹⁰. This requires condensation of the gaseous air to liquid and removal of the permanent diluent gases under vacuum at liquid nitrogen temperature. The calibration procedure involved a static dilution technique which has been described in detail elsewhere¹¹. The detection limit was

~ 0.5 p.p.t.v. (parts per 10^{12} by volume) for CH_3Br , 0.2 p.p.t.v. for CH_2Br_2 and CF_3Br and ~ 0.1 p.p.t.v. for CHBr_3 . The precision of the analysis and sampling procedure was $\sim 10\%$ for all four compounds as the largest error arose in estimating the volume used in the analysis stage.

Data for the variation of concentration with latitude are shown in Fig. 1 for CH_3Br , CH_2Br_2 , CHBr_3 and CF_3Br . In the case of methyl bromide (CH_3Br), (Fig. 1a) there is a clear reduction in concentration between the two hemispheres, such that the average concentration in the Northern Hemisphere (NH) is 15.4 ± 1.9 p.p.t. and that in the Southern Hemisphere (SH) is 10.6 ± 0.9 p.p.t. There is also more scatter in the limited amount of Northern Hemisphere data while the variation in the southern data is very close to the limits of precision for the GC/MS system ($\sim 10\%$). A similar interhemispheric gradient has been observed previously¹² but the concentrations observed in both hemispheres were higher.

Methylene dibromide (CH_2Br_2) (Fig. 1b) also shows a marked hemispheric difference in concentration (NH = 2.70 ± 0.59 p.p.t.v., SH = 1.58 ± 0.26 p.p.t.v.) with again less variation in the Southern Hemisphere. The bromoform (CHBr_3) data (Fig. 1c) are much more scattered than either the CH_3Br or the CH_2Br_2 data. Although there is a tendency for the Northern Hemisphere concentrations to be higher, the mean interhemispheric difference is within the scatter of the data. The values for the mean and standard deviation are NH = 0.85 ± 0.44 p.p.t.v., when the value of 5.96 p.p.t.v. at 20°N is omitted, and SH = 0.58 ± 0.30 p.p.t.v. with the inclusion of the equatorial value of 0.88 p.p.t.v. These are both valid procedures since; first, the CHBr_3 concentration in the 20°N sample was elevated far above the average whereas none of the other trace gases measured showed such an elevation, and second, other trace gases such as ethane and some saturated hydrocarbons in the equatorial sample gave results typical of the Southern Hemisphere. There is no evidence that the high value at 20°N was associated with long-range transport of a plume of anthropogenic pollutants, because other trace gases indicative of pollution were present at levels similar to the average for the Northern Hemisphere samples. The slightly higher concentrations of CHBr_3 at 75°S in 1982 and 50°S in 1983 cannot be explained in terms of anthropogenic emissions. These data could mean that CHBr_3 has oceanic sources in both hemispheres and that it is more reactive in the atmosphere than are the mono- and di-bromo-substituted methanes. The CHBr_3 data contrast with those for CF_3Br (Fig. 1d) which show a very small gradient north and south on the 1982–83 voyage and a variability well within the limits of precision of the measurements (NH = 1.15 ± 0.05 p.p.t.v., SH = 1.06 ± 0.09 p.p.t.v.). CHBr_3 and CH_2Br_2 have been identified in coastal seawater (S. A. P. and R. A. Rasmussen, unpublished data).

The different variability in the concentration of the four molecules is associated with different source distributions and reactivity. The simplest behaviour is that of CF_3Br (CFC-13B1) which is used as a flame retardant and is unlikely to have any natural sources. The molecule is mostly emitted into the atmosphere in the Northern Hemisphere and it has no significant tropospheric sinks. However, it is removed in the stratosphere by photolysis, as shown by measurements of its vertical profile up to 25 km (ref. 13). The small interhemispheric gradient is very similar to that of CFC 11 and 12, which have been growing in concentration at a rate close to $5\% \text{ yr}^{-1}$. A similar growth rate is therefore anticipated for CF_3Br . This compound was originally detected in the atmosphere in 1980 and its concentration then was estimated to be about 0.7 p.p.t.v. (ref. 10). Unfortunately, this was not a precise estimate and thus no absolute growth rate can be ascertained from our data at present.

CH_3Br is extensively used as a fumigant and annual production in 1972 was estimated to be about 300,000 tons, a quarter of which (75,000 tons) was probably emitted to the atmosphere¹. It has also been proposed that the ocean is a major source, as in the case of methyl chloride and methyl iodide^{13,14}.

If it is assumed that CH_3Br is mostly removed from the

atmosphere by reaction with hydroxyl radicals in the troposphere, then the present data can be used to estimate the size of the annual input. A recently calculated value of the global average hydroxyl concentration is 4×10^5 molecules cm^{-3} (ref. 15); the atmospheric burden of CH_3Br is about 2.26×10^5 tons according to our data. These data require an annual input of 90,000 tons assuming removal only by hydroxyl radical chemistry with a rate constant of 3.2×10^{-14} cm^3 per molecule per s at 280 K (ref. 15). This is only slightly higher than the estimate³ of anthropogenic atmospheric emission made in 1972 of 75,000 tons, suggesting either that the ocean makes a relatively small contribution to the atmospheric burden of CH_3Br or that our rather simple chemistry is incorrect. An independent check on the chemistry can be made by comparing the methyl bromide levels in the two hemispheres. Assuming only a Northern Hemisphere source for CH_3Br which is transferred to the Southern Hemisphere with a relaxation time of 400 days, then the global average hydroxyl concentration required to account for the data is 3.6×10^5 molecules cm^{-3} , assuming all reactions take place at 280 K. The chemistry, therefore seems to be consistent and this leaves a problem with the oceanic source of CH_3Br . An estimate made recently¹² suggests that 300,000 tons of CH_3Br are emitted annually from the ocean. This would far outweigh the anthropogenic emission of CH_3Br and would argue for the globally-even distribution in concentration, observed for CH_3Cl (ref. 12), which has a similar removal rate from the atmosphere by hydroxyl radicals¹⁶. The global average hydroxyl concentration is an uncertain quantity and a larger total emission of methyl bromide into the atmosphere can be allowed, but the weight of evidence does not favour a large natural source.

Our interpretation of the data, therefore, suggests that the predominant source of CH_3Br in the atmosphere is anthropogenic rather than natural. This has important consequences for stratospheric ozone chemistry. It has been assumed previously that the stratospheric burden of bromine was mainly natural in origin and that any reduction in ozone produced by its presence was not a recent phenomenon¹⁰. This assumption is now questionable, and we suggest that stratospheric bromine chemistry should be examined more carefully in the context of possible effects of increased anthropogenic emissions.

The degree of variation in concentration suggests that both CH_2Br_2 and CHBr_3 are quite reactive in the troposphere, more so than CH_3Br and much more so than CF_3Br . They may have a mixture of sources since the CH_2Br_2 and CHBr_3 concentrations have been observed to increase during pollution incidents in the Arctic¹. Also, GC/MS studies of sea water containing different types of seaweed collected at a coastal site in southern England, show evidence for the presence of many different bromine compounds. These included $\text{C}_2\text{H}_5\text{Br}$, $\text{C}_3\text{H}_8\text{Br}$, CH_2BrCl , CHBr_2Cl and at least an order of magnitude greater quantities of CH_2Br_2 and CHBr_3 (S. A. P. and R. A. Rasmussen, unpublished data). It seems possible from the following calculations that CHBr_3 is the most important natural vector for gaseous bromine in the atmosphere and that CH_2Br_2 also has substantial natural sources. Their chemical lifetimes are unknown at present because of lack of published data either on rates of removal by hydroxyl radicals or on their photochemistry. However, the rates of reaction of the bromine compounds CH_3Br and $\text{BrCH}_2\text{CH}_2\text{Br}$ with hydroxyl radicals are very similar to those for their chlorine analogues. It is not unreasonable to assume, therefore, that to a first approximation, the rates for CH_2Br_2 and CHBr_3 are also similar to those for their chlorine analogues, for which published data exist: $k_{280\text{K}} = 1.128 \times 10^{-13}$ cm^3 per molecule per s for CH_2Cl_2 and 8.28×10^{-14} cm^3 per molecule per s for CHCl_3 (ref. 16). These rates should lead to a substantial interhemispheric gradient such as that observed for CH_2Cl_2 in our own unpublished data, where Southern Hemisphere values are only a quarter of the Northern Hemisphere values. The concentration distribution shown in Fig. 1 thus argues strongly for substantial sources of both CH_2Br_2 and CHBr_3 in the Southern Hemisphere, which must surely be natural rather than anthropogenic.

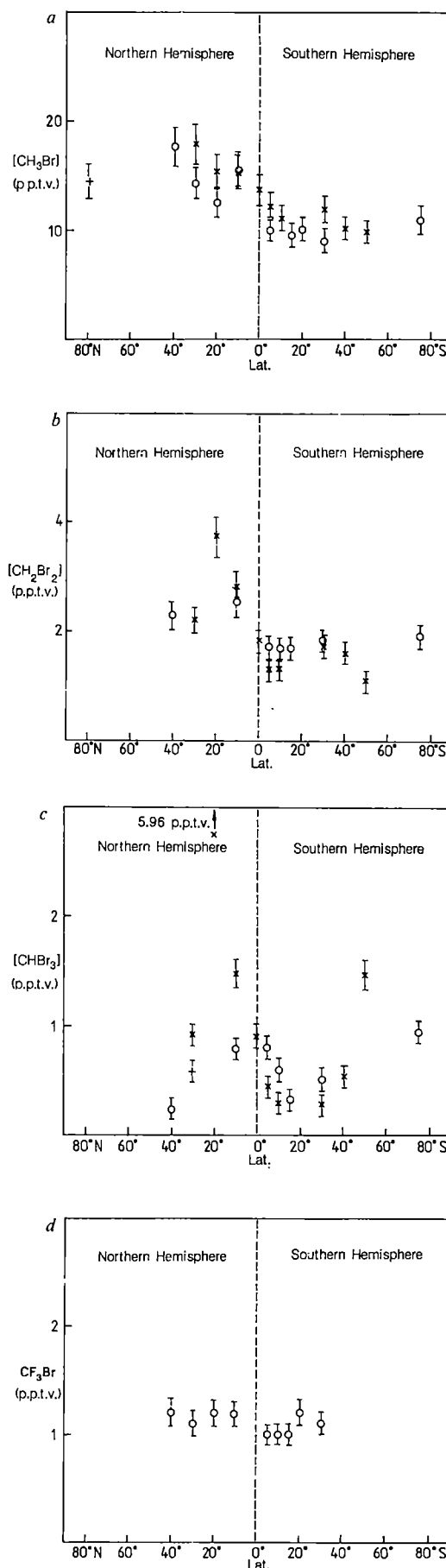


Fig. 1 Longitudinal transects of the concentrations of four bromine compounds: a, CH_3Br ; b, CH_2Br_2 ; c, CHBr_3 ; d, CF_3Br . \circ , 1982 voyage via Cape Town; \times , 1983 voyage via Rio; $+$, values collected on other experiments.

The case for natural sources of CHBr_3 is emphasized when a consideration is made of its potential atmosphere photochemistry. No published absorption spectrum could be found but some preliminary measurements were made at Harwell using a diode array spectrometer in conjunction with R. A. Cox. The absorption maximum occurs at a wavelength of 221 nm, where $\sigma = 5.51 \times 10^{-18} \text{ cm}^2$ per molecule and a significant overlap occurs in the region of tropospheric solar flux (305–320 nm). Assuming a quantum yield of unity, the atmospheric lifetime of CHBr_3 due to tropospheric photolysis could be as low as 2 weeks. This would require an annual emission in the region of 10^6 tons, which almost certainly exceeds the anthropogenic emissions many times over and makes CHBr_3 the most significant source of bromine released into the atmosphere. It is unlikely, though, that much CHBr_3 would penetrate into the stratosphere with a tropospheric lifetime this short.

Finally, there appear to be a substantial number of bromine compounds present in sea-surface waters that can find their way into the atmosphere. This indicates the presence of a rich bromine chemistry and mixed chlorobromo chemistry in the ocean that merit investigation in detail. The chemical behaviour of the bromine atoms released in both the troposphere and the stratosphere also needs study.

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Pyroxene solid solution in garnets included in diamond

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In what proved to be a temporary phase of trial mining of the Monastery Mine kimberlite pipe, South Africa, we recently had a brief opportunity to characterize the mineral inclusions in diamonds from this locality. The usefulness of such studies in elucidating mantle processes has already been demonstrated^{1–3}. We describe here garnets included in diamonds which have a unique range of compositions. They are interpreted as representing the first natural occurrence of garnet hosting a component of pyroxene in solid solution. Their discovery fulfils predictions based on successful laboratory experiments in which pyroxene was dissolved in garnet at very high pressures (50–180 kbar)^{4–6}. As this solid solution is accompanied by an increase in density, such results have both geophysical and chemical implications for the structure

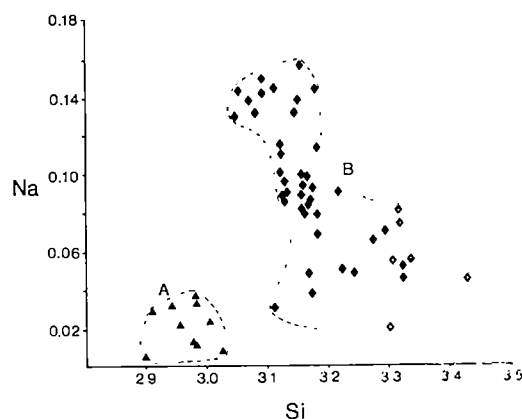


Fig. 1 Plot of atomic Na against atomic Si for 12 oxygens. Group A represents garnets of similar composition to eclogitic diamond inclusion garnets worldwide^{1–3,12–16}. Group B represents garnets interpreted to host a component of pyroxene in solid solution. Δ , Group A garnets; \diamond , group B garnets.

of the mantle. In addition, the pressures inferred are higher than generally proposed for mantle minerals sampled by kimberlites. Recent work has demonstrated that at least some inclusion-bearing diamonds have been derived from Archaean lithosphere⁷. The Monastery garnets in diamonds may indicate that the old lithosphere was very thick^{8,9}. Alternatively, if they have an asthenospheric origin, they may prove particularly interesting because of their different source.

Monastery Mine is situated near the border between South Africa and Lesotho, close to the inferred eastern margin of the Kaapvaal Craton. It was an inviting target for a study of mineral inclusions in diamonds because it is geographically well separated from the established kimberlite diamond mines in southern Africa and because it is a kimberlite with an abundance of fresh xenoliths and xenocrysts of mantle rocks and minerals to which the inclusions and hence the diamonds might be related. The general geology of Monastery has been described elsewhere¹⁰.

In this study, 131 inclusion-bearing diamonds in the size range $-9+5$ (ref. 11) were selected from 4,600 carats of general production. The diamonds are <3 mm in largest dimension and average ~ 0.12 carats per stone. After careful visual inspection and rigorous cleaning, the inclusions were liberated from the diamonds by mechanical crushing in an enclosed steel cracker. The individual inclusions, usually between 75 and 250 μm in longest dimension were mounted in epoxy resin on glass slides and polished. Mineral compositions were determined by means of a Cameca Camebax microanalyser, using standards of essentially similar composition to the unknowns and an on-line ZAF correction procedure. Analyses are believed to be accurate to $\pm 1\%$ relative and have been checked at two independent microprobe laboratories.

The minerals found as inclusions in diamonds from the Monastery Mine kimberlite are almost entirely of an eclogitic paragenesis. Fifty-six eclogitic garnets have been analysed. Each analysis represents an average of at least five individual spots either on a single inclusion or several inclusions from the same diamond. Two distinct populations of eclogitic garnets are present in the diamonds (Fig. 1). One group (termed group A) consists of 10 garnets which have compositions similar to those described from diamonds worldwide^{1–3,12–16}. They have Na_2O concentrations which range from 0.04 to 0.25 wt%, variable quantities of FeO , MgO and CaO typical of eclogitic garnets included in diamond, and SiO_2 and Al_2O_3 present in normal stoichiometric proportions, namely 39.0–42.2 wt% SiO_2 and 20.19–23.06 wt% Al_2O_3 (Fig. 2). The second group (group B) consists of 46 garnets that form a related suite of compositions which have never been reported before. Particularly extreme concentrations of Si and Al range to as high as 47.43 wt% SiO_2 and as low as 11.29 wt% Al_2O_3 (Fig. 2). Note that the low Al_2O_3 values are not supported by high Cr_2O_3 concentrations as occurs for peridotitic garnets, the maximum chrome concentration in

Table 1 Representative analyses of garnets included in diamonds

No.	1	2	3	4	5	6
Sample (Group)	A4-05 (A)	A4-03 (B)	B9-17 (B)	Al-20 (B)	Al-24 (B)	S-6
SiO ₂	41.76	42.08	43.45	45.43	47.43	46.11
TiO ₂	0.72	1.21	1.15	0.81	0.78	0.46
Al ₂ O ₃	22.45	18.32	16.30	14.82	11.29	15.64
Cr ₂ O ₃	0.29	0.01	0.06	0.06	0.20	0.43
FeO	9.29	14.74	12.99	9.77	10.51	8.04
MnO	0.23	0.27	0.27	0.29	0.19	—
MgO	20.60	10.31	13.48	18.20	22.05	20.88
CaO	4.31	11.89	11.93	9.27	7.11	8.16
Na ₂ O	0.08	1.08	0.64	0.58	0.33	—
Total	99.73	99.92	100.27	99.23	99.89	99.72
Cation proportions based on 12 oxygens						
Si	2.983	3.154	3.216	3.316	3.429	3.300
Ti	0.039	0.068	0.064	0.044	0.042	0.025
Al	1.890	1.618	1.422	1.275	0.962	1.320
Cr	0.016	—	0.004	0.003	0.011	0.024
Fe	0.555	0.924	0.804	0.596	0.635	0.481
Mn	0.014	0.017	0.017	0.018	0.012	—
Mg	2.193	1.152	1.487	1.980	2.376	2.227
Ca	0.330	0.955	0.946	0.725	0.551	0.626
Na	0.011	0.157	0.092	0.082	0.046	—
M*	3.089	3.188	3.329	3.382	3.608	3.334

Values given are for diamonds from Monastery Mine (analyses 1–5), while analysis 6 represents a garnet hosting a component of pyroxene in solid solution, synthesized experimentally at 146 kbar and 1,200 °C (ref. 6)

* M = Mg + Fe + Ca + Na.

these garnets being 0.37 wt% Cr₂O₃. Sodium can be present in extremely high concentrations, ranging from 0.14 to 1.03 wt% Na₂O (Fig. 2). Two garnets of similar composition to the group B garnets were reported from Jagersfontein by Tsai *et al.*¹⁴ but they did not comment on their unusual chemistry.

Representative analyses of both groups of garnets together with an analysis of an experimentally synthesized garnet⁶ are presented in Table 1. At first glance the extreme compositions in group B resemble amphibole compositions. However, samples spanning the compositional range of the garnets have been analysed by X-ray diffraction using a 57.4-mm Gandolfi camera and Co K α radiation. Extremely high-quality photographs were obtained and all unequivocally confirm a garnet structure for both groups of garnets. Cell parameters (a_0) calculated according to the equation

$$a_0^2 = (h^2 + k^2 + l^2)d_{hkl}^2$$

where a_0 is the unit cell parameter, hkl is the Miller index for a crystal plane, and d is the d -spacing of the crystal, and using the (10, 4, 0) reflection, range between 11.564 and 11.629 Å, which correlates well with cell parameters for pyrope, almandine and grossular (11.455, 11.53 and 11.85 Å respectively).

The pyroxene and garnet mineral families have closely related basic chemical formulae in that both possess cation to oxygen anion ratios of 2:3. Under appropriate conditions of pressure and temperature, small quantities of garnet may be taken into solid solution in pyroxenes^{17,18}. As pressure is increased, however, this solid solution breaks down into low-alumina pyroxene plus exsolved garnet¹⁹. Experimental evidence has demonstrated that the inverse of this reaction is also possible, namely the solution of pyroxene in garnet^{4–6,19,20}, and because of the large density difference involved, this reaction would also be expected to be strongly pressure dependent. Ringwood²⁰ demonstrated that as pressure increases, the solubility of pyroxene in garnet increases. Akaogi and Akimoto⁵ studied pyroxene–garnet solid solution equilibria over a wide range of temperatures and pressures in Mg- and Fe-rich systems. They found that the solubility of enstatite (MgSiO₃) in pyrope (Mg₃Al₂Si₃O₁₂) increases gradually from 60 to 140 kbar and then increases suddenly in the pressure range 140–175 kbar,

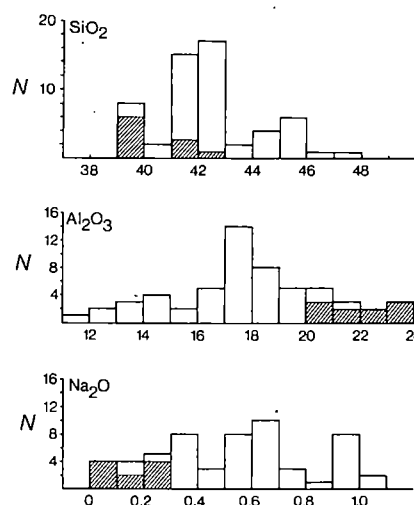


Fig. 2 Histogram of SiO₂, Al₂O₃ and Na₂O concentrations (wt%) in eclogitic diamond inclusion garnets from Monastery Mine. Shaded areas represent group A garnets (see Fig. 1 and text).

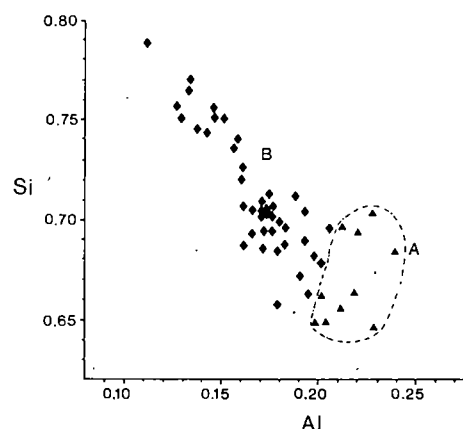


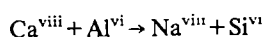
Fig. 3 Plot of Si (molecular proportion) against Al (molecular proportion) for eclogitic diamond inclusion garnets from Monastery Mine. Symbols as for Fig. 1.

resulting in the formation of a homogeneous garnet with a composition Mg₃(Al_{0.8}Mg_{0.6}Si_{0.6})Si₃O₁₂. Similar results were obtained for the ferrosilite–almandine system. The results of the experimental studies in simple systems have been verified and extended by Akaogi and Akimoto⁶, who successfully demonstrated a continuous increase in the solubility of pyroxene in garnet in a natural garnet lherzolite assemblage (PHN-1611) in the pressure range 45–205 kbar at temperatures of 1,050 and 1,200 °C. On the basis of these studies, Akaogi and Akimoto^{5,6} predicted that pyroxene would begin to dissolve in garnet at depths of 150 km in the upper mantle and that this would be manifested by an appreciable increase in the number of Si ions over the ideal value of 3 for 12 oxygens, indicating accommodation of Si ions in octahedral coordination.

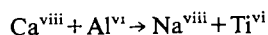
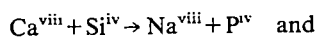
The group B diamond inclusion garnets at Monastery are interpreted in the light of the above experimental data. The number of Si ions for 12 oxygens in these garnets ranges from 3.046 to 3.429 (Fig. 1, Table 1), necessitating the existence of some Si in octahedral coordination. It is suggested that the group B garnets host a component of pyroxene in solid solution, an interpretation strongly supported by a comparison of the compositions of the group B garnets and the experimentally synthesized garnets⁶ (see Table 1, in particular analyses 4 and 6). Further evidence in support of this interpretation is the fact that the value of M (M = Mg + Fe + Ca + Na) in the group B garnets increases concomitantly with the increase of Si from 3.0 (see Table 1). Although M is not a good indicator of the presence of pyroxene in solid solution in garnet compared with Si, an increase of both M and Si is required to form garnet solid solution with pyroxene components (since pyroxene ~ Si + M).

Note that the Ca-rich garnets tend to have lower Si contents than the magnesian garnets, indicating that they have less pyroxene in solid solution. This is consistent with the data of Akaogi and Akimoto⁶, who found that systematically higher pressures are required for the solution of pyroxene in garnet in Ca-rich systems than in Fe- and Mg-rich systems. In addition, the Fe-Ca-rich group B garnets show extreme enrichment in Na and lesser depletions of Al than the magnesian group B garnets. This may be due to a similar effect to that described for inclusions in diamonds from Orapa³, where the composition of the pyroxene molecule in equilibrium with garnet changes with garnet composition. The major change is an increase in the jadeite molecule at the expense of diopside as iron and calcium in garnet increases. Solid solution of such pyroxenes in eclogitic garnets would be expected to produce the trends noted above.

Sobolev and Lavrent'ev²¹ suggested that elevated concentrations of Na in garnet may be correlated with high pressure. They proposed that Na enters the garnet structure according to the equation



thereby requiring some Si to be present in octahedral coordination. Thompson²² reported 0.06–0.57 wt% P_2O_5 in garnets synthesized from anhydrous basaltic melts at 18–45 kbar and 1,200–1,450 °C and proposed a coupled substitution between Na–P and Ca–Si. He points out that since the sum of atomic radii of Na and P is less than that of Ca and Si, the substitution of an Na–P couple into the garnet structure would be enhanced by high pressure. Bishop *et al.*²³ favour the idea that Na in garnets from peridotites and eclogites can be explained by substitutions of the type



without the need to invoke octahedral Si.

The group B garnets have been carefully analysed for P and found to have <0.01 wt% P_2O_5 . The coupled substitution between Na–P and Ca–Si is thus not feasible in this case. The coupled substitution between Ca–Al and Na–Si proposed by Sobolev and Lavrent'ev²¹ is also not possible, because of the existence of a negative correlation between Si and Na in these garnets (Fig. 1). The excellent negative correlation observed between Si and Al (Fig. 3), however, probably indicates that the substitutional scheme proposed by Akaogi and Akimoto⁶ to account for the compositions of their experimentally synthesized garnets (for example, Table 1, analysis 6) is also applicable to these garnets. The scheme involves the substitution of two Al^{3+} ions in the octahedral sites of the garnet structure by M^{2+} and Si^{4+} ions from the pyroxene structure ($\text{M}^{2+} = \text{Mg}^{2+}$, Fe^{2+} and Ca^{2+}). Na may be accommodated in the eight-coordinated dodecahedral site, as suggested by Sobolev and Lavrent'ev²¹.

The highest reported sodium value in an eclogitic diamond inclusion garnet was until recently 0.26 wt% Na_2O (ref. 21), but Hall and Smith²⁴ have reported Na-rich eclogitic garnets from Argyle, the highest concentration being 0.52 wt% Na_2O . Although they also failed to find a correlation between Na, Ti and P, appreciable P_2O_5 was detected and there is no evidence in the published analyses for excess silica or a deficiency of aluminium. Their garnets do not, therefore, appear to be like those described here.

The discovery of garnets from the upper mantle hosting pyroxene in solid solution and having Si in octahedral coordination is extremely important in view of current petrological modelling of the mantle. A controversial issue is whether the eclogite to garnetite transition is responsible for the 400-km seismic discontinuity^{25–27}. Our data show that the dissolution of pyroxene in garnet does occur in the upper mantle and, therefore, strengthens the evidence for the feasibility of the garnetite transition.

It is generally believed that diamonds have crystallized at depths >150 km (ref. 1), but the temperatures of equilibration calculated for coexisting diamond inclusion pairs^{1,12,13,24} are generally so low that origins deeper than 200 km have not been suggested. Palaeogeothermal reconstructions^{28,29} based on xenolith mineral compositions from kimberlite terminate at similar depths, which could mark the lithosphere–asthenosphere boundary. The group B garnets range to compositions showing extensive pyroxene solid solution in garnet, which is not seen in other mantle-derived garnets. As this solid solution is a predominantly pressure-dependent reaction, it seems likely that the group B garnets have an extremely deep origin. Based on experiments by Akaogi and Akimoto⁶ on a natural garnet lherzolite, pressures well above 100 kbar are possibly required; for example, garnet 6 in Table 1 was synthesized at 146 kbar. Note, however, that the pressure required to allow pyroxene solubility in garnet is substantially lower in Fe-rich systems than in Mg- and Ca-rich systems⁶. As the garnets under discussion are reasonably Fe-rich, the pressures required for their synthesis should not be as high as those required to produce pyroxene–garnet solid solution in the natural garnet lherzolite⁶. Therefore, no restricted range of likely depths of origin can be justified on the available evidence. Note that as a group 1 kimberlite¹⁰ the Monastery diatreme has an asthenospheric signature, and the diamonds with group B inclusions are therefore not constrained to a lithospheric origin in the absence of any knowledge of their isotopic characteristics.

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Forest changes in the Amazon Basin during the last glacial maximum

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We report here the discovery of forest beds in the Amazon Basin radiocarbon dated to the Wisconsin–Würm glaciation. These are the first dates from the last glaciation determined on Amazonian samples. The locality is in Ecuador, at 1,100 m elevation, near the upper limit of the present Amazonian rainforest. Both pollen spectra and *Podocarpus* wood in the deposits suggest that vegetation comparable to the present Andean forest grew at least 700 m lower than it does now, suggesting a temperature depression of at least 4.5 °C for the Amazon lowlands.

The forest beds are exposed along road cuts at two sites near Mera (1°28' S, 78°6' W, elevation 1,100 m) in Oriente Province, Ecuador (Fig. 1). The area has a mean annual temperature of 20.8 °C and precipitation of >4,800 mm, making it one of the wettest places in the Amazon Basin¹. The present vegetation is tropical forest of high diversity, at the transition between lower montane rainforest and the true lowland rainforest which begins at ~800 m elevation².

At the first exposure, an organic bed ~2 m thick is overlain by ~12 m of largely inorganic deposit (Fig. 2). Unsorted angular boulders both above and below the organic layer are embedded in a clastic matrix, suggesting lahars, though this must be confirmed by further field work. A darker lithological unit, possibly organic, overlies the upper debris flow and is in turn overlain by a fluvial sequence of clast-supported gravel and sand.

The organic bed has woody twigs or roots up to 10 cm in diameter running through it, has a loss-on-ignition ranging from 30 to 64%, and has high pollen concentrations. It thus appears to be a marsh or soil deposit that accumulated before burial under a debris flow. Two wood samples were collected *in situ* from the forest bed (samples 1 and 5) and sediment samples were collected at 0.3-m intervals for pollen analysis. Wood sample 5 yielded a radiocarbon date of $33,520 \pm 1,010$ yr BP (B-9618).

The second exposure lies about 3 km east of the first. The road-cut section is ~20 m high here and consists of deeply weathered fluvial sand at the base, overlain unconformably by a clast-supported gravel with rounded boulders. The forest bed, ~2 m thick, lies above the gravel and is overlain by fluvial sand. It could not be reached to obtain samples, but slumped blocks containing parts of tree trunks and stumps up to 30 cm in diameter at the foot of the section had apparently fallen from the fossil forest bed. This wood was embedded in a clay-supported matrix of angular pebbles. Wood samples numbers 2–4 were taken from the slumped blocks. Sample 4 has a radiocarbon age of $26,530 \pm 270$ yr BP (B-10170).

Wood samples from both forest beds were submitted to R. B. Miller (USDA Forest Products Laboratory, Madison) for identification. Wood samples 1, 2 and 4 are soft wood from conifers, although poor preservation precludes more precise identification. *Podocarpus* is the only conifer genus now occurring naturally in Ecuador³. *Pinus*, the conifer next nearest to Ecuador, grows no nearer than Nicaragua, at least 1,000 km away⁴. It is therefore reasonable to conclude that wood samples 1, 2 and 4 are of *Podocarpus*. There are indications that sample 1, from the first site, is of a different species from the 6,000-yr younger samples 2 and 4 of the second site.

Wood samples 3 and 5 are of hardwoods, sample 5 possibly of the genus *Tovomitia* (Guttiferae), a genus with both Andean and Amazonian species in the modern flora of Ecuador.

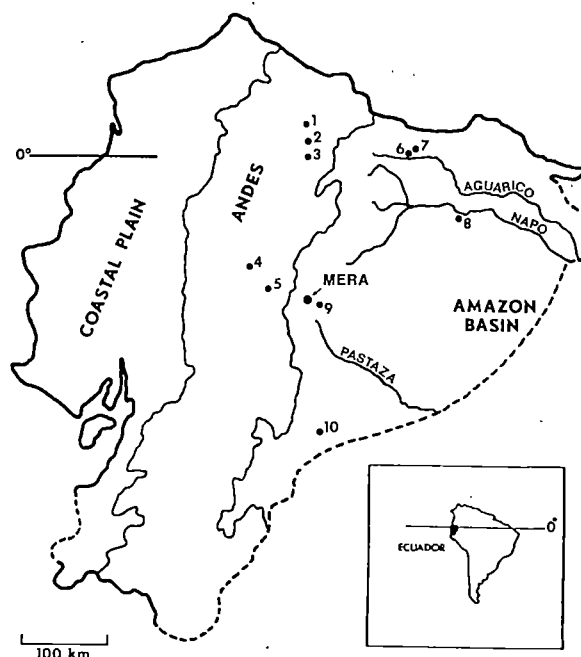


Fig. 1 Regions of Ecuador showing the Mera site of the Pleistocene forest beds and of the suite of surface samples. 1, Lake Conru; 2, Lake San Marcos; 3, Yaguarcocha; 4, Lake Yambo; 5, Rum Tum; 6, Lake Sta. Cecilia; 7, Lake Agrio; 8, Añangucocha; 9, Puyo Bog; 10, Lake Kumpak⁵.



Fig. 2 Mera forest bed: first exposure. Arrows mark edges of polleniferous organic layer containing woody parts of *Podocarpus* dated at $33,520 \pm 1,010$ yr BP.

Pollen concentration (calculated by the exotic pollen technique) of 660,000 grains per cm³ in a sample of the organic bed at site 1 is almost an order of magnitude higher than concentrations typical of the Andean and Amazonian lake samples of Fig. 1. Table 1 compares the percentage pollen spectra from this sample with 10 surface or recent spectra from Ecuador, 5 from the Andes and 5 from the Amazon forest. The sample from Puyo

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Table 1 Pollen from Mera forest bed compared with modern pollen spectra

	Conru (2,800 m)	Yambo (2,600 m)	Yaguarcocha (2,210 m)	San Marcos (3,400 m)	Rum Tum (2,392 m)	Kumpak ^a (700 m)	Añangucocha (230 m)	Puyo Bog (953 m)	Lago Agrio (330 m)	Sta Cecilia (330 m)	Mera (1,100 m)
<i>Podocarpus</i>		0.3	0.4								0.4
Palmae				0.6				0.3	0.6	1.0	
<i>Iriartea</i>					0.3	2.3	2.4	0.3	13.3	9.3	1.2
<i>Cecropia</i>	0.7	2.3	0.4			43.1	63.4	43.3	39.3	49.8	0.8
<i>Ficus</i>						3.4		1.2	7.2	1.0	
Urticaceae-Moraceae	5.2	10.6	4.4	1.7	2.0	10.4	4.8	17.3	14.5	14.1	4.0
Melastomaceae	1.4	3.0	1.6	1.0	1.7	1.9		1.5	0.9	0.3	7.6
<i>Trema</i>		0.7		0.7		0.4		1.2	2.3	2.3	
<i>Piper</i>						1.9	1.6	0.6	3.5	0.7	
<i>Alnus</i>	2.1	1.0	0.4	0.3							4.8
<i>Hedyosmum</i>	0.3			0.3							10.0
<i>Acalypha</i>						13.7		0.9	0.9	2.3	
Gramineae	36.8	27.3	14.0	37.7	70.0	1.5		6.3			0.8
Compositae	1.7	2.3	2.4	5.7	4.3		0.8	2.7	0.3	1.0	0.8
<i>Plantago</i>	3.8	2.0				0.4					
<i>Rumex</i>	8.3	1.0	1.6								
Chen-Am	1.7	8.7	7.8		0.3	0.4	0.8				
Cyperaceae	27.4	5.7	57.8	2.7	1.3		1.6	1.5			
Monolete	0.7	2.0	0.8	11.3	2.3	2.7	10.6	2.7	1.7	1.6	13.6
Trilete	0.9	3.0	2.4	5.0	0.3	2.3		1.3		2.3	2.8
'Others'	12.5	31.9	18.7	29.6	11.6	21.0	27.3	19.5	18.9	14.3	53.2

Sites shown on Fig. 1. Añangucocha sample is from surface pinches of moist forest soil; Puyo Bog is surface organic matter of a tropical swamp; Rum Tum is mud from a small pond and Yaguarcocha is from just below the settlement layer of a sediment core to yield pollen from the Interandean Plateau before human disturbance. Remaining samples are from mud-water interfaces of lakes. We distinguish >200 pollen taxa in Amazonian fossil pollen samples, although many of these cannot yet be given taxonomic names. We have included as 'others' many identifiable, but infrequent pollen types as well as a considerable number of tricolporate and tricolpate taxa yet to be identified. We have also combined subdivisions of other taxa like Moraceae and Urticaceae.

Bog is from within 5 km of site 1 in the same forest type. Table 1 shows that the pollen rain falling at Mera 33,000 yr ago is comparable to pollen of high elevations in the modern Andes and not to pollen emanating from the lowland rainforest like that falling near Mera today. The inclusion of significant percentages of the Andean taxa *Alnus* and *Hedyosmum* is particularly striking, as is the virtual absence of the *Cecropia* found at high percentages in every sample we have yet examined from Holocene and surface samples from Ecuadorian Amazonia, including the immediate vicinity of Puyo.

The Pleistocene pollen assemblage is Andean, although not closely comparable to pollen from any modern facies of the Andean forests. For instance, the taxa Melastomataceae, monolete fern spores, and *Hedyosmum* are in proportions not closely matched by the Andean spectra available. One explanation of an unusual pollen assortment would be pollen transport and redeposition by streams mixing pollen from different elevations, but the very high pollen concentration and organic matrix of the deposit make it unlikely that the beds represent stream deposits. This conclusion is supported by the presence of *Podocarpus* megafossils (twigs or roots) embedded in the polleniferous organic matrix of site 1. The *Podocarpus* megafossils are unlikely to have been redeposited in a richly polleniferous organic sediment by rapid mountain streams. The data thus suggest that Andean forest including *Podocarpus* grew at 1,200 m elevation at times during the last glacial cycle.

Podocarpus typically grows today in the Andean forest between the 2,000- and 3,500-m contours in Ecuador and Colombia^{2,3,5,6}. Four species occur naturally in Ecuador: *P. montanus* (including *P. montanus* var. *densifolius*), *P. oleifolius*, *P. glomeratus* and *P. sprucei*. Altitudinal ranges given in the literature are 2,000–2,800 m for *P. oleifolius*, 2,500–4,000 m for *P. glomeratus* and 2,000–3,000 m for the whole section of *Stachycarpus*^{3,5}. These reports suggest that 2,000 m is about the lowest altitude at which populations of *Podocarpus* are to be expected. The lowest confirmed modern elevation in the records of the Fundacio Natura of Ecuador is 1,800 m.

The late Pleistocene populations of *Podocarpus* grew at least 700 m below the lowest modern populations. If, as seems likely for mountain conifers, the altitudinal ranges are temperature dependent, we can calculate the necessary temperature depression. Assuming a lapse rate of 0.65°C per 100 m, the minimum lowering of mean annual temperature is 4.5°C. As the pollen data suggest extensive montane forests at this eleva-

tion, the actual lower limit of *Podocarpus* was certainly below 1,100 m and the actual temperature depression >4.5°C. This conclusion is compatible with earlier estimates of a temperature depression of 6°C on the altiplano of Colombia⁶. These new data, however, are the first to demonstrate temperature depressions of this magnitude in the Amazon lowlands themselves.

Note that both our radiocarbon dates fall within various definitions of a mid-Wisconsin interstade⁷, suggesting that forest and temperature depressions at full glacial times were even larger than our calculations suggest. There are no data which could establish the extent to which an interstadial was expressed along the American Equator, although an episode of glacial advance younger than 25,000 yr ago has been documented from the Ecuadorian Andes⁸. It is likely that the displacement of vegetation and climate during the glacial maximum at 18 K was even larger than that suggested by the Mera data.

These data are of particular interest in view of the hypothesis that much of the Amazon Basin was arid during times of glacial maxima, with the rainforest reduced to several large refugia in regions of highest modern rainfall^{9–11}. Prominent among suggested refugia is the region between the Napo and Pastaza rivers in which the Mera sites lie, since this is the Amazonian region of highest contemporary rainfall. Our pollen data show that at least part of the territory of this supposed refugium in fact supported forest comparable to modern, montane Andean forest from 33,000–26,000 yr BP in Wisconsin–Würm times.

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Calcium gradients in single smooth muscle cells revealed by the digital imaging microscope using Fura-2

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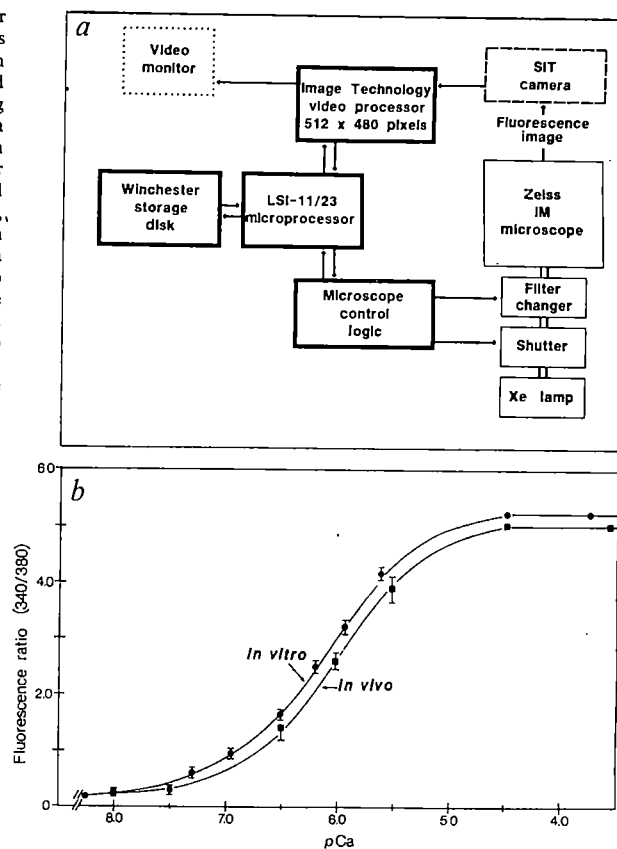
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Calcium is believed to control a variety of cellular processes, often with a high degree of spatial and temporal precision. For a cell to use Ca^{2+} in this manner, mechanisms must exist for controlling the ion in a localized fashion. We have now gained insight into such mechanisms from studies which measured Ca^{2+} in single living cells with high resolution using a digital imaging microscope and the highly fluorescent Ca^{2+} -sensitive dye, Fura-2. Levels of Ca^{2+} in the cytoplasm, nucleus and sarcoplasmic reticulum (SR) are clearly different. Free $[\text{Ca}^{2+}]$ in the nucleus and SR was greater than in the cytoplasm and these gradients were abolished by Ca^{2+} ionophores. When external Ca^{2+} was raised above normal in the absence of ionophores, free cytoplasmic Ca^{2+} increased but nuclear Ca^{2+} did not. Thus, nuclear $[\text{Ca}^{2+}]$ appears to be regulated independently of cytoplasmic $[\text{Ca}^{2+}]$ by gating mechanisms in the nuclear envelope. The observed regulation of intranuclear Ca^{2+} in these

contractile cells may thus be seen as a way to prevent fluctuation in Ca^{2+} -linked nuclear processes during the rise in cytoplasmic $[\text{Ca}^{2+}]$ which triggers contraction. The approach described here offers the opportunity of following changes in Ca^{2+} in cellular compartments in response to a wide range of stimuli, allowing new insights into the role of local changes in Ca^{2+} in the regulation of cell function.

The resting Ca^{2+} distribution in smooth muscle was determined using a digital imaging microscope to measure the fluorescence of enzymatically disaggregated toad (*Bufo marinus*) stomach cells¹ loaded with Fura-2². This cell type has been studied in detail (for review see ref. 3), previous work having revealed that, like other types of smooth muscle, these cells use Ca^{2+} from different sources for different stimuli⁴⁻⁶. The recording system (shown schematically in Fig. 1a) was developed to detect light within a single cell with extremely high accuracy, sensitivity and resolution, so that a very small number of molecules could be detected in a limited region of a cell⁷. $[\text{Ca}^{2+}]$ at each point in a cell was determined by measuring the fluorescence intensity (500 nm, emission) with excitation at 340 nm and at 380 nm² (Fig. 2a, b). The 340-nm image (Fig. 2a) showed large regional inhomogeneities in fluorescence intensity which reflected both Ca^{2+} and dye distribution, and which were also influenced by cell geometry. Division of this image, on a pixel-by-pixel basis, by that obtained with 380 nm (Fig. 2b) resulted in a ratio image (Fig. 2c) in which variations in fluorescence intensity within the cell were indicative² of spatial variation in $[\text{Ca}^{2+}]$. The nuclear and sub-plasmalemmal fluorescence detail,

Fig. 1 Acquisition of Fura-2 cellular fluorescence images and determination of cellular $[\text{Ca}^{2+}]$. **a**, Schematic illustration of digital imaging microscope used to acquire and process images of cellular Fura-2 fluorescence. Fluorescence of Fura-2-loaded single smooth muscle cells was imaged using a Zeiss Ultrafluor 100 \times , NA = 1.25 objective and an inverted microscope (Zeiss IM) equipped for epifluorescence. Video images were obtained using a silicon-intensified target camera (Dage MTI, model 66), with the output digitized to a resolution of 512 \times 480 pixels by an Image Technology video analyser with each point in the image being assigned a value from 0 to 255 depending on intensity. The shutter/filter changer were obtained from Digital Microscope Accessories Co. The optics and digital imaging system provide better than 0.25 μm resolution in a given image plane. However, the large depth of focus of the fluorescent microscope results in information from a much greater depth than this being present in a given image plane⁷. For example, light from a point source 2 μm either above or below a given specimen plane will be attenuated to only 30% at the position in the image corresponding to the point source. Images were stored on a high-capacity Winchester disk (20 megabyte capacity) and digitally analysed using a PDP-11/23 microcomputer. Multiple video frames were sequentially added to memory to increase the signal-to-noise ratio in the averaged image. To correct for spatial variations in dark current of the camera, averaged images, obtained without input to the camera, were subtracted from the images on a pixel-by-pixel basis. To correct for spatial variations in the intensity of the excitation source, as well as spatial variations in camera gain, the image was multiplied pixel-by-pixel with a 'system gain normalization' map. This map was constructed by obtaining an image of a uniform fluorescent field (a well-mixed solution of Fura-2 free acid), with intensities at each point being divided by the mean fluorescence intensity so that the gain normalization did not change the mean grey level of an image. Images of Fura-2 fluorescence at 500 nm emission were obtained with 340 and 380 nm excitation wavelengths using filters from Dittic Optics Inc. (Hudson, Massachusetts) (emission filter, 500 nm, Halfwidth, 20 nm; excitation filters, 340 nm, Halfwidth, 10 nm; 380 nm, Halfwidth, 10 nm). The ratio image ($R_{340/380}$) was achieved from the division of the 340-nm image by the 380 nm image on a pixel-per-pixel basis. A fluorescence intensity threshold was set before the division to exclude non-cellular regions from the determination of the ratio image. We have not attempted to interpret information in pixels immediately adjacent to the cell edge due to a low signal-to-noise ratio, and the extreme sensitivity of the ratio image at these points to small mechanical and/or electronic shift between the two images. All images were photographed using a Celtic Technology video/filter recorder. **b**, Relationship between Fura-2 fluorescence ratio and $[\text{Ca}^{2+}]$. *In vitro* calibration: smooth muscle cells were loaded with Fura-2 (Fura-2/AM) for 90 min. The external loading solution (amphibian Krebs Ringer or a similar HEPES-based buffer) was removed by gentle centrifugation (~ 200 r.p.m.) and replaced by a solution very like the composition of the intercellular milieu: (mM) K^+ , 100; Na^+ , 30; Mg^{2+} , 1; ATP, 5; Balanced Electrolyte Solution, 20. Small aliquots of cells (300 μl) were each exposed to different external $[\text{Ca}^{2+}]$. The $p\text{Ca}$ of the bathing solution was controlled by varying the ratio of Ca-EGTA/EGTA, and was determined by potentiometric titration of free and total (~ 10 mM) EGTA concentrations by CaCl_2 and CdSO_4 , respectively^{25,26}. This solution also contained 1–2 μM ionomycin to allow equilibration of intra- and extracellular Ca (ref. 6). Higher concentrations of ionomycin (3–5 μM) can completely remove the external cell membrane in this cell type. Fluorescence ratios (340/380) were obtained from several individual cells from each aliquot. Absolute measured ratios are displayed as a function of the calculated $p\text{Ca}$ of the external solution. $[\text{Ca}^{2+}]$ determined using this calibration curve coincided closely ($\pm 5\%$) with values determined using the standard calibration equation (Fig. 3 legend). Possible differences in dye properties due to binding to highly immobile cellular sites were investigated by determining the fluorescence emission anisotropy ($r = (I_{\parallel} - I_{\perp}) / (I_{\parallel} + 2I_{\perp})$)²⁷ under the microscope (380 nm excitation). Fluorescence anisotropy values within different cellular compartments (cytoplasm, 0.08 ± 0.01 , $n = 11$; nucleus, 0.08 ± 0.02 , $n = 6$) were not significantly different from those produced by free dye in solution (0.05 ± 0.01 , $n = 8$). Much higher anisotropies (up to 0.34) are observed when the dye is embedded in highly viscous solutions in cuvettes. *In vitro* calibration: Fura-2 pentapotassium salt (50 μM) was added to small volumes of buffer (mM): K-MOPS, 10; 100; Mg^{2+} , 1; $\text{K}_2\text{H}_2\text{-EGTA} + \text{Ca-EGTA}$ 10; pH 7.20) and sealed in Lucite wells. Each volume contained a different $[\text{Ca}^{2+}]$ attained and measured as described above. Ratios were determined under the digital imaging microscope from fluorescence intensities at 340 and 380 nm excitation corrected for the background fluorescence obtained in the absence of Fura-2.



evident particularly in the 340 nm image, was still present in the ratio image, indicating that it reflects localized areas of higher $[Ca^{2+}]$, rather than simple differences in dye concentration.

The precise relationship between the fluorescence ratio (340/380) and free $[Ca^{2+}]$ in the intracellular environment was determined by exposing cells to solutions of varying $[Ca^{2+}]$ in the presence of levels of Ca^{2+} -ionophore previously shown⁶ to result in equilibration of intracellular and extracellular $[Ca^{2+}]$. We found that the fluorescence ratio varied in response to changes in cellular $[Ca^{2+}]$ in a manner virtually identical to that of free dye in solution (Fig. 1b). The *in situ* calibration curve was used in turn to calculate $[Ca^{2+}]$ at each point within the cell (as described in Fig. 3). Figure 3a shows the resulting map of spatial variations of $[Ca^{2+}]$ within this cell. Intracellular variations in $[Ca^{2+}]$ are displayed as a solid surface whose height at each pixel is proportional to pCa . This image shows quite strikingly that Ca^{2+} is not uniformly distributed within the smooth muscle cell, but is present at higher concentrations inside the nucleus than in the cytoplasm. It is also present at higher concentrations in sub-sarcolemmal sites whose distribution is remarkably similar to what has been presumed to be SR in other smooth muscles⁸. Further support for the assumption that these regions reflect $[Ca^{2+}]$ inside the SR comes from observations of Fura-2 fluorescence in skinned smooth muscle cells. In saponin-skinned cells, where the SR is intact, areas of high $[Ca^{2+}]$ along the cell margins were apparent, whereas in cells skinned with Triton X-100, which permeabilizes both sarcolemma and SR, these areas were no longer detectable (G. J. Kargacin, D.A.W. and F.S.F., unpublished observations). Mean $[Ca^{2+}]$ over any region in these cellular maps was calculated using interactive graphics software to define the boundaries of the selected regions. Cytoplasmic $[Ca^{2+}]$ in 45 cells was 137 ± 13 (s.e.m.) nM, which compares favourably with other determinations made in the same cell type using other techniques (129 ± 4 nM ($n=22$), as measured from quin2 fluorescence in cell suspensions⁶ and 163 (148 with Na^+ correction) ± 20 nM ($n=16$) using a Ca -sensitive microelectrode; H. Yamaguchi, per-

sonal communication). Nuclear $[Ca^{2+}]$ in resting smooth muscle cells was significantly higher than cytoplasmic, averaging 245 ± 20 nM in 13 cells and being 220 nM in the cell shown in Figs 2 and 3a. Note that when $[Ca^{2+}]$ is calculated from measurements of total cell fluorescence (for example, as in fluorometric measurements using cells in suspension), fluorescence contributions from the nucleus and SR would result in an overestimate of cytoplasmic $[Ca^{2+}]$ by 8–10%. This source of contamination can be significantly reduced by using shorter dye loading times to reduce dye entry into the SR (see also Fig. 2 legend).

The observation that free $[Ca^{2+}]$ within the cell nucleus appears to be higher than in the cytoplasm is an unexpected finding which might be explained if intranuclear $[Ca^{2+}]$ were regulated by nuclear membrane-dependent processes, or may represent an artefact resulting from modified behaviour of the dye in the nuclear environment. This latter explanation seems unlikely in the light of several findings. One finding is that the dye inside the nucleus has the same rotational freedom (as measured by fluorescence emission anisotropy) as that in the cytoplasm, suggesting that the dye is not bound to highly immobile intracellular sites (see Fig. 1b). An even more direct test of this possibility was obtained in experiments where cells were exposed to the Ca^{2+} -selective ionophore ionomycin, which resulted in collapse of the $[Ca^{2+}]$ gradient between nucleus and cytoplasm, as well as between SR and cytoplasm (see Fig. 3b). This was also seen in ionomycin-treated cells in which cytoplasmic $[Ca^{2+}]$ was maintained below normal by incubation of cells in external $[Ca^{2+}]$ levels below 1.8 mM. The maps derived from the fluorescence ratio (340/380) revealed no differences between nucleus, SR and cytoplasm, although the higher dye content of the nucleus was still clearly evident in non-ratio images (not shown). Finally, while not all cells so far studied in this manner (human neutrophils, mouse lymphocytes, BALB/c 3T3 cells) exhibit a higher $[Ca^{2+}]$ in the nucleus than in the cytoplasm, all these cells did exhibit different intranuclear $[Ca^{2+}]$ from that of the cytoplasm, suggesting that Ca^{2+} regulation at the nuclear-cytoplasmic interface may be a general cellular property.

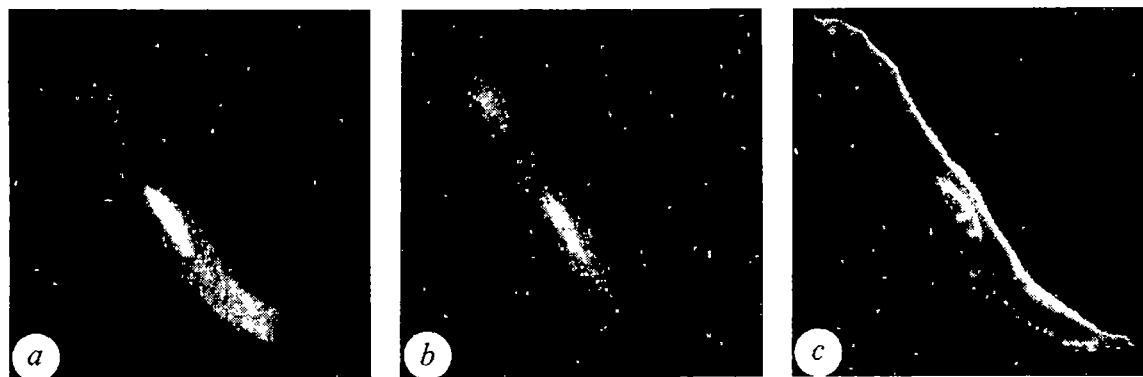


Fig. 2 Images of Fura-2 fluorescence in a single smooth muscle cell. *a*, 340 nm excitation, 500 nm emission; *b*, 380 nm excitation, 500 nm emission; *c*, ratio 340/380 image. Cell length, 150 μ m; maximum cell diameter, 11 μ m. All images were obtained as described in Fig. 1a legend. The fluorescence image displayed in *a* indicates a large degree of intracellular heterogeneity in fluorescence intensity, reflecting regional differences in dye and Ca^{2+} concentration and also that the dye can enter subsarcolemmal sites (SR). The ratio image (*c*) indicates that the cellular ionized $[Ca^{2+}]$ is apparently higher in the SR and nucleus. To determine if the high $[Ca^{2+}]$ measured over the nucleus merely reflects high $[Ca^{2+}]$ within the perinuclear space projected, because of the large depth of focus of the fluorescence microscope, onto the profile of the nucleus, we digitally calculated the image to be expected for this possibility. We assumed that intranuclear and cytoplasmic $[Ca^{2+}]$ were equal and that the perinuclear space was 0.2 μ m wide, containing a 10-fold higher $[Ca^{2+}]$. Nuclear dimensions were those determined from the cell displayed in *a–c*. The expected image was determined by convolving this model cell with the point spread function of the optical system which was determined as described previously⁷. A profile of $[Ca^{2+}]$, determined midway through the nucleus of the resultant image (results not shown), differed considerably from that observed in the actual image in that $[Ca^{2+}]$ in this image was higher along the periphery of the nucleus than over its centre. Just the opposite pattern was actually observed. A similar procedure was used to assess the contribution of SR and nuclear fluorescence to $[Ca^{2+}]$ measured in the cytoplasm. The cell was modelled as a cylinder, 150 μ m long with SR distributed in an outer annulus 1 μ m wide, encircling a cylinder of cytoplasm 10 μ m diameter. The nucleus (2 μ m wide, 10 μ m long) occupied a central position. Fluorescence intensities in each model area were calculated from the mean values in Fig. 2 at each wavelength. This model was convolved with the point spread function of the microscope. In the mid-focal plane of the model, at points adjacent to and significantly away from the nucleus, calculations revealed that the $[Ca^{2+}]$ would be overestimated by $\sim 15\%$ and 3% in these two locations, respectively, due to contribution of out-of-focus information from non-cytoplasmic sites.

Methods. Fura-2⁸ was introduced into these cells by incubating small aliquots (300 μ l) of cells in amphibian physiological saline (APS) at 30 $^{\circ}$ C with 0.3 μ M Fura-2/AM (the acetoxymethyl ester form of the dye). After 60–90 min of incubation, cellular esterases had cleaved and entrapped 20–100 μ M of the working form of the dye (as determined fluorometrically in cell suspensions), suitable concentration for monitoring most physiological processes under investigation. Substantial dye loading into the SR could be achieved by simply increasing the duration of the loading phase to ~ 180 min and is probably enhanced by the peripheral location of the SR in this cell type. The images displayed here and in Fig. 3 were all obtained after loading for 180 min (cytosolic Fura-2 concentration 150–160 μ M). Dye which had been loaded and cleaved by smooth muscle cells displayed spectral properties characteristic of free dye (acid) in solution².

Fig. 3 Maps of the spatial variation in $[Ca^{2+}]$ in smooth muscle cells. Profiles of pCa in a relaxed smooth muscle cell (*a*, from Fig. 2c) and an ionomycin-treated cell in the presence of normal (1.8 mM) extracellular calcium (*b*). Vertical heights at each pixel in these images are proportional to the pCa at that location within the cell. The areas of high intensity evident in the ratio image (Fig. 2c), the nucleus and subsarcolemmal sites, (presumably the SR), are clearly apparent in *a*. Average $[Ca^{2+}]$ in each of these cell areas are: cytoplasm, 130 nM; nucleus, 220 nM; SR, 500 nM. The $[Ca^{2+}]$ gradients between the cytoplasm, nucleus and SR are no longer evident in ionomycin-treated cells.

Methods. Fluorescence ratio images were converted to $[Ca^{2+}]$ using the equation $K_D[(R - R_{min})/(R_{max} - R)]\beta = [Ca^{2+}]$,

where R is the measured 340/380 ratio at each point in the cell, R_{max} and R_{min} are the fluorescent ratios of the same point in the cell, for a well-mixed solution of Fura-2 + Ca^{2+} and Fura-2, respectively, and β is the ratio of fluorescence at 380 nm excitation for Fura-2/Fura-2 + Ca^{2+} . The K_D of the intracellular dye was determined from the data in Fig. 1b to be 200 nM. Note that the $[Ca^{2+}]$ calculated by determining the ratio on a point-by-point basis (as above) is not equivalent to that determined by taking the ratio of the average fluorescence of large areas within the cell as the latter method would tend to underestimate the real mean $[Ca^{2+}]$ because of the non-distributive aspect of the calculation as well as the nonlinear relation between the 340/380 fluorescence ratio and $[Ca^{2+}]$. $[Ca^{2+}]$ values were converted to pCa and displayed as a solid surface on a Lexidata Model 3400 Imageview/Solidview Graphics System. These images were constructed using a standard surface modelling system which represented the cell surface as a group (160,000) of triangular polygons. Random high-frequency noise was removed by filtering (or convolving) the image with a gaussian filter whose width at half-height was 3 pixels.

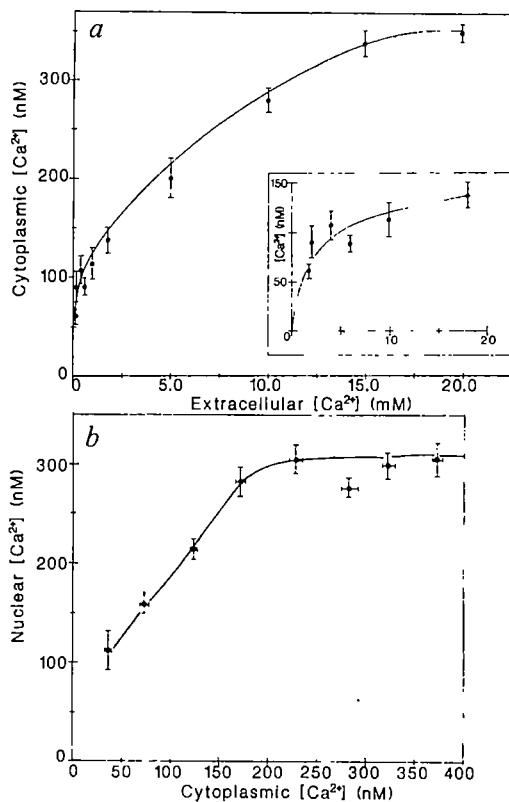
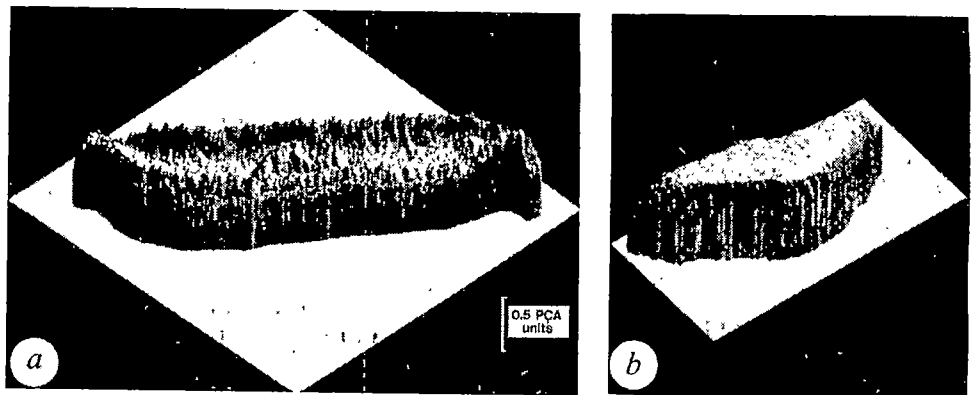


Fig. 4 Dependence of cytoplasmic and nuclear $[Ca^{2+}]$ on extracellular and cytoplasmic $[Ca^{2+}]$, respectively. *a*, Cytoplasmic $[Ca^{2+}]$ was determined in randomly selected areas of multiple smooth muscle cells as a function of external $[Ca^{2+}]$. Results are expressed as mean \pm s.e.m. with a minimum of 10 observations for each point. The inset shows, on an expanded scale, the relationship between cytoplasmic $[Ca^{2+}]$ and extracellular $[Ca^{2+}]$ below 2.0 mM. As can be seen, cytoplasmic $[Ca^{2+}]$ was relatively constant in the face of a large change in the transmembrane $[Ca^{2+}]$. The relative constancy of cytoplasmic $[Ca^{2+}]$ might reflect saturation of carrier-mediated Ca^{2+} influx, or the operation of a pumping mechanism with a high Hill coefficient (>2.8). This Hill coefficient is inferred from the observed dependence of cytoplasmic $[Ca^{2+}]$ on extracellular $[Ca^{2+}]$, assuming that Ca^{2+} permeability is fixed and that influx increases 36-fold at 0.5–18 mM extracellular $[Ca^{2+}]$ which, in the steady state, must be accompanied by a similar increase in Ca^{2+} pumping out of the cell. This requires that over the observed range of cytoplasmic $[Ca^{2+}]$ (130–350 nM), the Hill coefficient for Ca^{2+} pumping is 2.86, a high level of positive cooperativity. *b*, Dependence of nuclear $[Ca^{2+}]$ on cytoplasmic $[Ca^{2+}]$. The cytoplasmic $[Ca^{2+}]$ values determined in *a* were placed into 50-nM groupings (0–49, 50–99 ... 350–399) irrespective of the extracellular $[Ca^{2+}]$ which produced each cytoplasmic $[Ca^{2+}]$. These values were then averaged and the respective nuclear $[Ca^{2+}]$ values plotted as a function of average $[Ca^{2+}]$ for each cytoplasmic $[Ca^{2+}]$ range. All results are presented as means \pm s.e.m. Lines were fitted to the data points by eye.

The observation of a $[Ca^{2+}]$ gradient between both cytoplasm and extracellular space as well as between nucleus and cytoplasm suggests that both the plasma membrane and nuclear membrane have important Ca^{2+} -regulatory functions. To obtain further insights into the existence and nature of these processes, we exposed smooth muscle cells to solutions of varying extracellular $[Ca^{2+}]$ and determined the steady-state distribution of Ca^{2+} across the sarcolemma and nuclear membrane (Fig. 4). A strong dependence of cytoplasmic $[Ca^{2+}]$ on extracellular $[Ca^{2+}]$ was seen only below 0.5 mM (Fig. 4). By contrast, cytoplasmic $[Ca^{2+}]$ changed by no more than 3-fold as $[Ca^{2+}]_{out}$ was varied over a 36-fold range from 0.5 to 18 mM. This may be indicative of the involvement of a saturable carrier protein mediating Ca^{2+} influx in the plasmalemma or an outwardly directed Ca^{2+} pump having a large Hill coefficient (~ 3) between 130 and 350 nM $[Ca^{2+}]$ (see Fig. 4 legend). The existence of an inverse relation between Ca^{2+} 'permeability' and extracellular concentration has in fact been demonstrated previously for these cells⁶.

The changes in cytoplasmic $[Ca^{2+}]$ induced by varying extracellular $[Ca^{2+}]$ allowed us to observe the dependence of intranuclear $[Ca^{2+}]$ on cytoplasmic $[Ca^{2+}]$. As can be seen in Fig. 4b, although nuclear $[Ca^{2+}]$ closely followed changes in cytoplasmic $[Ca^{2+}]$ below the resting level (130 nM), it was virtually unaffected by increases in cytoplasmic $[Ca^{2+}]$ above this value, thereby revealing for the first time membrane-dependent mechanisms for regulating intranuclear $[Ca^{2+}]$. We doubt that higher intranuclear $[Ca^{2+}]$ is the simple result of a Donnan effect of excess negative charge in the nucleus relative to the cytoplasm. Ionomycin should not directly affect a Donnan gradient yet does abolish the observed nuclear-cytoplasmic difference. Furthermore, a Donnan effect would predict a constant nuclear to cytoplasmic free $[Ca^{2+}]$, in contradiction to the results shown in Fig. 4b, and should also influence the distribution of all permeant ions between nucleus and cytoplasm; however, studies of monovalent ion distribution by others^{9,10} indicate that any Donnan effect must be quite small. Finally, a Donnan potential that accumulates Ca^{2+} would repel Fura-2 and its Ca^{2+} complex (-5 and -3 charged, respectively), whereas the dye concentration is actually higher in the nucleus. The possibility that the nuclear membrane acts to regulate nuclear $[Ca^{2+}]$ may initially seem surprising given ultrastructural data revealing the existence of pores in this membrane¹¹, observations that dyes of relative molecular mass $<20,000$ rapidly enter the nucleus when injected into cells¹², and measurements of monovalent ion distribution between nucleus and cytoplasm revealing equivalent concentrations^{9,10}. On the other hand, there are data to support the proposal that the nuclear membranes regulate $[Ca^{2+}]$. First, the nuclear pores are not patent but filled

with electron-dense material arranged in an ordered manner¹¹. Second, transport functions are known to reside in the nuclear membranes—a system for active uptake of specific proteins has been extensively studied^{13–15} and, particularly germane to the present proposal, a Ca^{2+} -ATPase has been reported to exist in nuclear membranes^{16,17}. Finally, measurement of total Ca^{2+} with the electron microprobe, while not directly comparable to the present data on free Ca^{2+} , suggest that nuclear Ca^{2+} is somehow screened from the effect of increased cytoplasmic Ca^{2+} during contraction of rabbit portal-anterior mesenteric vein¹⁸, although screening is not apparent in the rabbit main pulmonary artery¹⁹.

The proposal that nuclear function may be subject to Ca^{2+} -linked regulation, as are many cytoplasmic processes, is certainly supported by recent observations revealing the presence of the Ca^{2+} -linked modulatory protein, calmodulin, within the nucleus of various cells²⁰, as well as *in vitro* observations revealing a Ca^{2+} or calmodulin sensitivity, for several known intranuclear

enzymes^{21–23} and for prolactin gene expression²⁴. It remains to be seen which nuclear processes are indeed regulated by Ca^{2+} *in situ* and if and when during cell activity nuclear $[\text{Ca}^{2+}]$ changes. The demonstrated ability to measure $[\text{Ca}^{2+}]$ with a high degree of spatial resolution in single living cells opens the exciting possibility of measuring changes in $[\text{Ca}^{2+}]$ in various cellular compartments (for example, cytoplasm, nucleus and SR) during changes in physiological activity to explore such questions.

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Sequences homologous to the P mobile element of *Drosophila melanogaster* are widely distributed in the subgenus *Sophophora*

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The biological properties of the P element of *Drosophila melanogaster* differ from those of other mobile elements in this genus in that its mobility is associated with dysgenic traits that might be expected to limit its distribution or spread¹. The P element is widely distributed among natural populations of *D. melanogaster*, but is absent from strains that have been maintained in the laboratory for more than thirty years². This observation prompted speculation that the P element has recently invaded and spread horizontally through *D. melanogaster*^{3,4}, a hypothesis supported by the observation that P elements are absent from the *D. melanogaster* sibling species⁵. However, sequences homologous to P elements have been found recently in *Drosophila paulistorum*, a quite distant relative⁶. This finding undermines any simple hypothesis about the origin of P elements, and demands a thorough analysis of the distribution and conservation of the P element within the subgenus *Sophophora*. We show here that the P element is present in every species group in the subgenus *Sophophora*, but that its distribution within species groups can be discontinuous. The P homologues from different species are not identical; furthermore, their genomic localization differs from that found in *D. melanogaster*.

D. melanogaster belongs to the subgenus *Sophophora*. This subgenus has 145 species distributed among four species groups: *melanogaster*, *obscura*, *saltans* and *willistoni*⁷. We examined 20 species from the subgenus, representing all 4 species groups.

The species chosen differ in their geographical range, habitat and relatedness. The divergence of the *melanogaster* group from both the *willistoni* and *obscura* species groups is estimated to have occurred about 50 million years ago⁸. Table 1 shows the phylogenetic relationships of the species used in this study. About 100 adult flies of each species were examined carefully under a dissecting microscope to confirm their identity, and DNA was prepared from them⁹, digested with *Pvu*II, separated by electrophoresis and transferred to nitrocellulose filters¹⁰. An internal 840-base-pair (bp) *Hind*III fragment from the *D. melanogaster* P element p π 25.1 was purified, labelled and hybridized to the filters (see Fig. 1). The distribution of P homologues within the subgenus under low-stringency wash conditions is shown in Table 1 (see Fig. 1 legend for details). All members tested of the *willistoni* and *obscura* groups have P-element homologues, whereas some species from the *melanogaster* and *saltans* species groups lack sequences with detectable homology to the P element. Previous investigators did not detect P homologues in *D. pseudoobscura*⁵, but this may be due to differences in the hybridization and wash conditions, or to intraspecies differences.

The filters were washed subsequently at increasing stringencies to estimate the degree of similarity between P homologues from the different species and the *D. melanogaster* P element. The representative data shown in Fig. 1 demonstrate that each species group has P-homologous sequences with distinct properties. P homologues from *melanogaster* species are weakly detectable only at low stringency (Fig. 1). More closely related P homologues are found in the *obscura* group species. The hybridization signal is detectable after low- and medium-stringency washes, but is undetectable after high-stringency washes. The most similar P homologues are found in the *willistoni* and *saltans* species groups, in which the signal is retained even when the filters are washed in $0.2 \times \text{SSC}$ at 63°C . Note that all *willistoni* species group members have a 0.9-kilobase (kb) *Pvu*II fragment that co-migrates with the 0.9-kb *Pvu*II fragment from the complete P element of *D. melanogaster*, whereas no member of the *saltans* group has such a fragment. This suggests that the P-homologous sequences differ between the *willistoni* and *saltans*

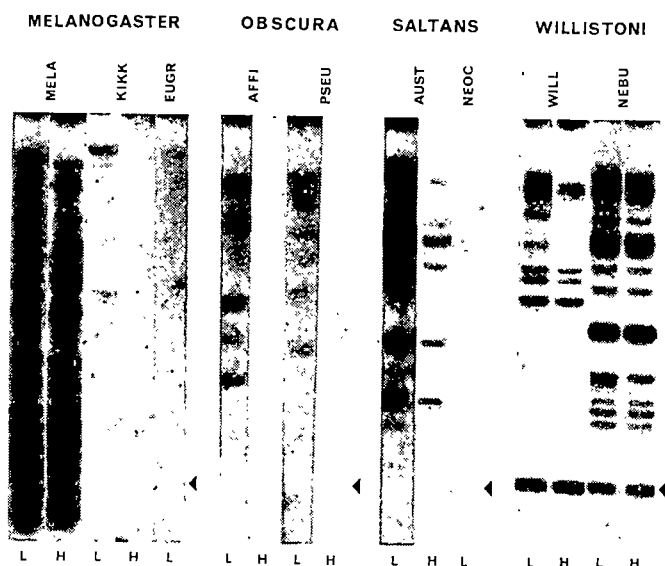


Fig. 1 Autoradiographs of Southern blots containing Sophophoran species DNA probed with P element DNA from *D. melanogaster*. DNA was prepared from adult flies using slight modifications of a published procedure⁹, digested with *Pvu*II, subjected to electrophoresis on 0.6% agarose gels and transferred to nitrocellulose¹⁰. The 840-bp internal 5' *Hind*III fragment of p π 25.1 was eluted from agarose gels, nick-translated¹⁵ to a specific activity of 0.5–1.0 \times 10⁸ d.p.m. μ g⁻¹ with ³²P-dCTP, and used as a probe. Hybridization conditions were 6 \times SSC, 50% formamide, 5 \times Denhardt's solution¹⁶, 10 mM EDTA, 0.5% SDS for 12–16 h at 37 °C. The filters were washed three times for 15 min each under the following conditions: low stringency (2 \times SSC, 0.1% SDS; room temperature); medium stringency (0.2 \times SSC, 0.1% SDS; 53 °C); high stringency (0.2 \times SSC, 0.1% SDS; 63 °C). The stringency used is indicated below each lane, using the following abbreviations: L (low) and H (high). The species groups and species are indicated above each gel lane. Abbreviations are as follows: mela (*D. melanogaster*), kikk (*D. kikkawai*), eugr (*D. eugracilis*), affi (*D. affinis*), pseu (*D. pseudoobscura*), aust (*D. australosaltans*), neoc (*D. neocordata*), will (*D. willistoni*) and nebu (*D. nebulosa*). The arrowhead indicates the position of a 0.9-kb *Pvu*II fragment which co-migrates with the internal 0.9-kb *Pvu*II fragment of p π 25.1.

groups. The most striking observation is that the P homologues from species more closely related to *D. melanogaster* show least homology to the *D. melanogaster* P elements. Representative P homologues have been cloned from *D. willistoni* and *D. nebulosa*, and have restriction maps very similar to the map of the P element of *D. melanogaster*. A 2.9-kb P homologue from *D. nebulosa* shows extensive DNA sequence conservation compared with p π 25.1 (R.A.L. *et al.*, manuscript in preparation).

The apparent differences among P homologues prompted us to examine the location of these sequences within their respective genomes by *in situ* hybridization. In *D. melanogaster*, the P element can insert at many positions within the euchromatic portion of the genome, and although it can insert into heterochromatin, it is rarely found there^{11,12}. Figure 2 shows the results of *in situ* hybridization using either *D. melanogaster* internal P-element restriction fragments, or using P-homologue sequences cloned from *D. saltans* or *D. nebulosa*. In each of *D. nebulosa*, *D. saltans* and *D. willistoni*, the probe hybridizes extensively to centromeric heterochromatin. Only in *D. willistoni* did we regularly detect at least two euchromatic sites of hybridization. This pattern contrasts with the predominantly euchromatic location of P elements in *D. melanogaster*.

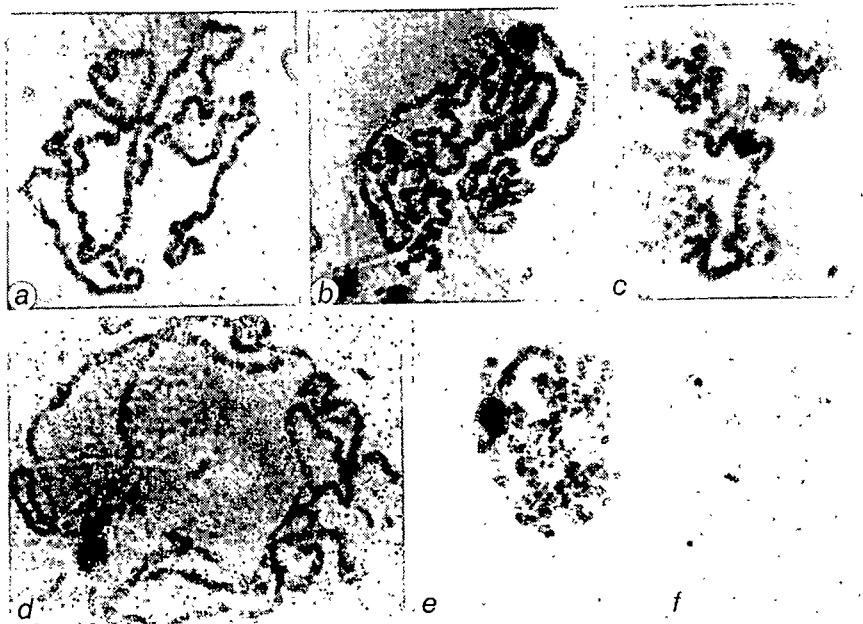
The observation that P homologues are detectable in all species groups of the Sophophoran subgenus might lead one to conclude that the P element is evolutionarily ancient, and that it appeared before the Sophophoran radiation. However, discontinuous distribution of the P homologues within species groups suggests that P elements have invaded some species after the Sophophoran radiation. If the element existed prior to the Sophophoran radiation, then the discontinuous distribution must result from the loss of the P element from some populations, but the observation that P homologues from species most closely related to *D. melanogaster* are the least conserved relative to the *D. melanogaster* P element strongly argues against a single ancient origin of the P element. The fact that P homologues are widespread but not identical in the Sophophoran subgenus suggests that Drosophilids have been subject to multiple invasions at undetermined times from an unknown external source, or alternatively, that P elements occasionally move horizontally from within the genus. The latter suggestion implies that species bearing P homologues should now have, or have had in the past, overlapping ranges. This prediction is supported by the observation that P homologues are present in species

Table 1 Phylogenetic relationships among Sophophoran species used, and distribution of P homologues

Subgenus	Species group	Subgroup	Species	P homologues
Sophophora	melanogaster	ananassae	ananassae	—
		montium	kikkawai	+
		elegans	elegans	—
		eugracilis	eugracilis	—
		takahashi	takahashi	—
		ficuspshila	ficuspshila	+
		obscura	pseudoobscura	+
		affinis	affinis	+
		willistoni	willistoni	+
			equinoxialis	+
			tropicalis	+
			paulistorum	+
			paulistorum-like	+
			nebulosa	+
	saltans	succinea	succinea	+
		capricornii	capricornii	+
		saltans	saltans	+
		australosaltans	australosaltans	+
		prosaltans	prosaltans	+
		sturtevantii	sturtevantii	+
		elliptica	emarginata	—
		cordata	neocordata	—

The table shows the species group, subgroup and species of each species used in this study, taken from Throckmorton⁷. All stocks were obtained from the National *Drosophila* Species Resource Center, Bowling Green State University, Ohio. The right-hand column shows whether P homologues were detected under our low-stringency wash conditions (see Fig. 1 legend).

Fig. 2 *In situ* hybridization of P elements to chromosomes of *Sophophora* species. DNA was labelled to a specific activity of $3\text{--}5 \times 10^7$ d.p.m. μg^{-1} with ^{35}S -dCTP and hybridized to polytene chromosomes¹⁷. Hybridization conditions were $5 \times \text{SSPE}$, 50% formamide, $2 \times$ Denhardt's solution¹⁶, $100 \mu\text{g ml}^{-1}$ sonicated salmon sperm DNA, at 35°C for 12–16 h. Slides were washed three times for half an hour each in $2 \times \text{SSC}$ at 50°C , dried through ethanol and prepared for autoradiography¹⁸. **a**, The complete P element p $\pi 25.1$ hybridized to *D. melanogaster*, showing exclusively euchromatic localization of the P elements. **b**, P homologue from *D. nebulosa* subcloned into pUC12, hybridized to chromosomes from *D. nebulosa*, showing chromocentric localization of P elements. **c**, A gel-purified internal fragment from the 3' end of the same *D. nebulosa* homologue used in **b**, hybridized to *D. nebulosa* chromosomes to demonstrate that flanking sequences do not account for hybridization to the chromocentre. **d**, A P-homologous sequence from *D. saltans* hybridized to chromosomes of *D. saltans*. Most grains are observed over the chromocentre. Similar results were obtained when the *D. nebulosa* internal P-element probe was hybridized to chromosomes from *D. saltans* (data not shown). **e**, Hybridization of 3' internal P-element restriction fragment from *D. nebulosa* to chromosomes of *D. willistoni*. Most hybridization is to the chromocentre, although two euchromatic sites can occasionally be detected. **f**, Hybridization of the 840-bp internal 5' *Hind*III fragment from p $\pi 25.1$ used for the genomic Southern analysis hybridized to chromosomes of *D. willistoni*. Two euchromatic sites of hybridization are detected and shown here, although a third site is visible after longer exposure.



whose ranges overlap the South American and Central American range of the *willistoni* species group¹³, like the *saltans* and *obscura* groups⁷, or the nearly cosmopolitan *D. melanogaster* and *Drosophila kikkawai*⁷. The absence of P elements from some species in our sample could have several origins. First, the P element or its as yet unidentified putative vector, could have a limited host range. Second, if the source of the P element is outside the genus *Drosophila*, the source may be limited in its geographical range, perhaps constrained by its primary host. Third, the populations sampled may simply be M strains, whereas other populations of the same species may contain P homologues.

The P homologues from all three species examined by *in situ* hybridization appear to be largely confined to the heterochromatin. This heterochromatic localization may arise because heterochromatin contains a hotspot for integration of P elements in these species. If P factors are not transcriptionally active because they are confined to heterochromatin, then it would not be expected that they would spread through the population. It is interesting that in reactive strains of the IR dysgenic system of *D. melanogaster*, I elements are confined to heterochromatic regions, where presumably they are non-functional¹⁴. Molecular and biological tests of P-homologue function will be required in order to understand the origin and evolution of P elements.

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Regulation of the distribution of *Ultrabithorax* proteins in *Drosophila*

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The role of the bithorax complex (BX-C) in the genome of the fruitfly *Drosophila* is to specify the developmental pathways followed by cells in most of the insect's thoracic and abdominal segments¹. The BX-C has three lethal complementation groups, *Ubx*, *abd-A* and *Abd-B*, which have their major effects in progressively more posterior parts of the animal (Fig. 1)². Within the *Ubx* region³ there are four phenotypically distinct classes of mutations, *abx*, *bx*, *bxl* and *pbx*, each of which results in a part of the *Ubx* mutant phenotype^{1,4}. Here we show that these subfunction mutations all affect the distribution of *Ubx* proteins. The results support the view that the *Ubx* proteins represent the executive functions of the *Ubx* region⁵ and that the subfunction mutations affect regulatory regions required for the normal pattern of *Ubx* expression^{5,6}. In addition, we show that the region of the BX-C containing the *abd-A* and *Abd-B* complementation groups regulates the distribution of *Ubx* products.

The phenotype of *Ubx* mutations primarily affects four compartments: the posterior second thoracic compartment (T2p), the anterior third thoracic compartment (T3a), T3p and the anterior compartment of the first abdominal segment (A1a). In *Ubx* mutants, the sequence T2p T3a T3p A1a is transformed to

How do the subfunction mutations effect these transformations? Molecular analysis of the proximal region of the BX-C has revealed two transcription units⁵. One of these, the *Ubx* unit, is known to produce several transcripts and at least three

In *bx*³ third thoracic leg disks, *Ubx* protein expression is dramatically reduced in the anterior compartment (Fig. 2I). A similar effect is seen in the anterior compartment of the third thoracic dorsal disks (data not shown). In *pbx* third thoracic

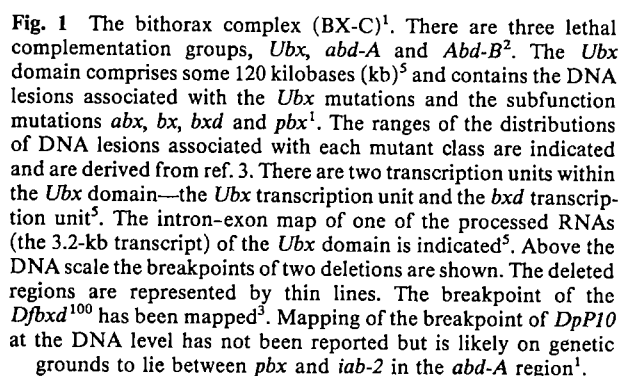


Figure 1 consists of 13 panels. Panels a, b, d, e, f, h, i, j, k, l, m, and n are fluorescence micrographs showing the development of ommatidia in the Drosophila eye. Panel g is a schematic diagram of an ommatidium, showing the arrangement of cells and the positions of the 'A' (accessory) and 'P' (pigment) cells. The panels are arranged in two rows: the top row contains a, c, e, g, i, k, m and the bottom row contains b, d, f, h, j, l, n. The micrographs show the progression from early stages (a, b) to later stages (m, n), with panel g providing a structural reference.

Methods. The immunofluorescence labelling of the disk whole-mounts has been described previously¹⁶. The disks were dissected from third-instar larvae and the homozygous mutant larvae were identified by using the balancer chromosome TM6B, constructed by L. Craymer and E. B. Lewis, which carries the dominant *Tb* marker. The *bx*³ homozygous third thoracic dorsal disks are not shown as they are necrosed.

ventral and dorsal imaginal disks, a complementary pattern is observed; there is a marked reduction in the level of *Ubx* protein expression in the posterior compartments (Fig. 2f, n). In neither case is there an effect on the labelling in the second thoracic disks. These results provide a molecular demonstration of the compartment-specific effect of the *bx*³ and *pbx* mutations and correlate with their compartment-specific effects on the adult cuticle^{11,12}.

The mutation *abx*² seems to resemble *bx*³ in its effect on *Ubx* protein expression in imaginal disks. In the third thoracic disks the most prominent effect is a reduction of the labelling in T3a in both the dorsal and ventral disks (Fig. 2d, j). However, the phenotype is more variable than *bx*³ and some disks exhibit patchy labelling in T3a and some dorsal disks also show patches of reduced labelling in T3p. This shows some correlation with the effects of *abx*² on the adult cuticle, where the most prominent effect appears to be a variable *bx*-like T3a → T2a transformation⁴. However, *abx* mutations also show a T2p → T1p transformation¹⁴ and thus the surprising aspect of the *Ubx* protein pattern in *abx*² is that this compartment seems to be unaffected.

Thus, these three subfunction mutations all affect the distribution of *Ubx* products in imaginal disks. The effects are correlated with the phenotypes of the mutants in the adult cuticle and may reasonably be supposed to be their cause. The mutations *abx*, *bx* and *pbx* therefore behave as if they affect regulatory functions required for the expression of *Ubx* products in specific areas. In the case of *bx* and *pbx*, these specific areas are T3a and T3p, respectively.

We have also examined the distribution of *Ubx* proteins in the nervous system, epidermis and mesoderm of mutant stage-13 embryos¹⁸. Here we will concentrate on the pattern of labelling in the ventral nervous system (VNS) as this is the most prominently labelled structure at this stage (Figs 3, 4). The wild-type pattern has been described previously^{5,16,17}. It exhibits a repeat unit, the *Ubx* metamere, which is out of phase with the segmental neuromere. The boundaries of the *Ubx* metameres may coincide with anterior/posterior compartment boundaries in the epidermis¹⁷ and thus *Ubx* metameres probably coincide with parasegments¹⁹. *Ubx* proteins are expressed in parasegments 5–13 in the VNS, with parasegment 6 being the most strongly labelled (Figs 4, 5).

In the embryos homozygous for the mutation *abx*², expression of *Ubx* products is reduced in parasegment 5 (Figs 4, 5). In the wild type the anterior region of parasegment 5 is more strongly labelled than the posterior. In *abx*² some labelling remains, particularly in the posterior region. However, our interpretation of the pattern is that the whole parasegment is affected. This agrees with the analysis of *abx* mutations on the commissure pattern in the VNS where *abx* mutations were observed to affect the pattern in both posterior T2 and anterior T3 (refs 14, 20).

The effect of *abx*² shows an interesting cell-type specificity. In the wild type the midline cells at the anterior edge of parasegment 4 (that is in T1) and those in T2 are labelled¹⁷. The labelling in T1 was unexpected, as the most anterior effect of *Ubx* mutations has been reported in the adult cuticle in T2p. The labelling pattern in the midline cells might be reconciled with the pattern in the rest of the VNS if the midline cells were to migrate one parasegment length anterior. In *abx*² the *Ubx* expression in the midline cells is unaffected (Fig. 4b). This result does not support the migration hypothesis, as in *abx*² there are two clusters of midline cells labelled anterior to the main block of labelling in the VNS. Rather, it indicates that the spatial control of *Ubx* expression in the midline cells differs from that in the rest of the VNS. Another example of cell-type specificity is the observation that *abx*² seems to have no effect on *Ubx* expression in the epidermis at this stage. The tissue-specific effect of *abx* over parasegment 5 suggests that it does not act to eliminate a compartment-specific product that is required to specify the T2p developmental pathway.

In contrast to the clear effect of *abx*², the mutations *bx*³ and *pbx* have no obvious effect on the distribution of *Ubx* products in either the VNS or the epidermis in stage-13 embryos. These

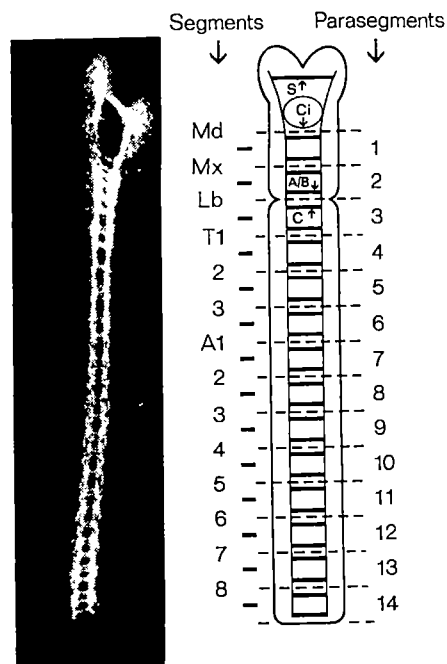


Fig. 3 Landmarks in the VNS—the commissure pattern. On the left is a dissected whole-mount of a stage-13 embryo labelled with fluorescein-coupled anti-horseradish peroxidase (HRP) antibody^{16,26}. This labelling reveals the pattern of transverse commissures (A/B and C) and lateral connectives in the VNS. On the right is an interpretation of the commissure pattern showing the segmental and parasegmental units. The head segments are represented by the supraoesophageal (S) and circular (Ci) commissures and posterior to this there is a repeat pattern of 14 parasegmental units^{17,19}. The boundaries of the parasegments are indicated on the right and probably coincide with the boundaries of the *Ubx* metameres in the VNS¹⁷. The boundaries of the *Ubx* metameres appear to lie on the anterior edge of commissure C (refs 16, 17). The segmental boundaries are indicated on the left and lie between commissure C and the succeeding A/B commissure²⁷. Md, mandibular; Mx, maxillary; Lb, labial.

mutations also have little or no effect on the commissure pattern in the VNS²⁰. We have also examined the *Ubx* pattern in the triple mutant *abx*¹ *bx*³ *pbx* and found that this shows a very similar pattern to *abx*². (In *abx*¹ *bx*³ *pbx* the reduction in *Ubx* expression appears to be more severe than in the *abx*². Whether this is due to the difference between the two *abx* alleles or to the influence of the *bx*³ and *pbx* mutations has not been investigated.) The lack of effect of the *bx* and *pbx* mutations at this stage contrasts with their clear effects on *Ubx* expression in imaginal disks and suggests that these mutations affect functions which are specifically required late in development or in the imaginal cell lineages.

While the subfunction mutations discussed above appear to reduce *Ubx* protein levels in rather specific locations, the effect of *Tpbxd*¹⁰⁰ extends over the whole range of the *Ubx* protein distribution⁵ (Figs 4, 5). Parasegments 5 and 6 are well labelled and, compared with the wild type, the labelling seems greater in the posterior part of parasegment 5 and less in parasegment 6. Parasegments 7–11 show an abnormal pattern, with fewer nuclei clearly labelled. There is a severe reduction in labelling from parasegment 11 to parasegment 12. In the wild-type a similarly severe reduction in labelling is seen between parasegment 12 and parasegment 13 (Fig. 5). This widespread effect of *bx*d mutations on *Ubx* expression correlates with their extensive phenotypic effects¹.

The second type of regulation that we have investigated is the influence on *Ubx* protein distribution of the centromere-distal region of the BX-C, containing the *abd-A* and *Abd-B* complementation groups. Deletion of this distal region in *DpP10;DfP9* homozygotes results in a transformation of parasegments 7–13 to parasegment 6 (refs 1, 10). If high-level *Ubx*

Fig. 4 *Ubx* protein expression in the VNS in wild-type and mutant stage-13 embryos. Dissected embryo whole-mounts were labelled with FP.3.38 to reveal *Ubx* proteins and with anti-HRP to reveal the commissure pattern. The parasegmental boundaries are indicated and are derived by superimposition of the anti-HRP pattern on the FP.3.38 pattern. *a*, Wild type. The *Ubx* proteins are expressed in parasegments 5–13. The most prominent expression is in parasegment 6. The labelling declines posteriorly and drops sharply in parasegment 13 where only four nuclei are well labelled at this stage. *b*, *abx*² homozygous embryo. The labelling is wild type apart from a reduction in parasegment 5. Note that the midline cells anterior to parasegments 4 and 5 are unaffected. *c*, *abx*¹, *bx*³ *pbx* homozygous embryo. Similar to *b* but slightly greater reduction of labelling in parasegment 5. *d*, *Tpbxd*¹⁰⁰. Altered pattern in parasegments 5–13. Note parasegments 5 and 6 are very similar. Compared with wild-type, fewer nuclei are clearly labelled in parasegments 7–11. Parasegment 12 shows a pattern similar to wild-type parasegment 13. *e*, Homozygous *DpP10; DfP9* embryo. This mutation leaves the *Ubx* domain intact but removes *abd-A* and *Abd-B* (see Fig. 1). Parasegment 5 is unaffected, but parasegments 6–13 exhibit the wild-type pattern of parasegment 6. *f*, *Pc*³ homozygous embryos. There is labelling over the whole VNS. Although labelling intensity declines from anterior to posterior, most parasegments exhibit a pattern similar to that seen in wild-type parasegments 7–12.

Methods. Embryos were dissected from heterozygous stocks and labelled as described previously¹⁶. The stocks and the fraction of embryos showing the mutant phenotypes illustrated above were: *abx*²/TM1, 8/41; *abx*¹ *bx*³ *pbx*/TM1, 12/36; *Tpbxd*¹⁰⁰/TM1, 11/42; *DpP10; DfP9*/TM1, 7/31; *Pc*³/TM1, 5/36. The above frequencies are all consistent with the expected 1/4 homozygous mutant progeny. Of 24 embryos from *bx*³/TM1 stocks and 29 embryos from *pbx*/TM1 stocks, none showed any clear deviation from the wild-type pattern.

expression is a prerequisite for the parasegment 6 developmental pathway, then in *DpP10; DfP9* homozygotes parasegments 7–13 should express high levels of *Ubx* protein, and this is indeed what we find (Figs 4, 5). Thus it appears that in the wild type, *abd-A* and/or *Abd-B* regulate *Ubx* protein distribution in parasegments 7–13. Such interactions between homoeotic genes have been proposed²⁸ and the regulation of the distribution of *Antennapedia* transcripts by genes in the BX-C has provided a clear example²¹. This interaction is investigated and discussed more fully elsewhere²².

We would like to note here how this interaction contributes to our understanding of other mutant effects. The first is tentative and concerns the *Tpbxd*¹⁰⁰ phenotype. In embryos homozygous for *Tpbxd*¹⁰⁰, the effect on *Ubx* product distribution shares two features with the *DpP10; DfP9* effect in that many segments are affected and the pattern is altered in both VNS and epidermis. In addition, the sharp decrease in *Ubx* protein level in the wild-type pattern in parasegment 13 occurs in parasegment 12 in the *Tpbxd*¹⁰⁰ pattern. The decrease in wild-type parasegment 13 is part of the regulation of *Ubx* product distribution by genes in the centromere-distal region of the BX-C, as it is not observed in *DpP10; DfP9* embryos (see also ref. 22). These features suggest that an important part of the effect of *Tpbxd*¹⁰⁰ on *Ubx* protein distribution may be its effect on the interactions between homoeotic genes.

The second case is the effect of *Polycomb* (*Pc*) on *Ubx* protein distribution. The labelling pattern in the VNS of homozygous *Pc*³ embryos is shown in Figs 4 and 5 and is similar to that reported previously⁵. The whole of the VNS is labelled, and although the intensity of labelling is quite variable, it generally tends to be higher anteriorly and to tail off posteriorly. The *Pc* gene has been postulated to act as a repressor of BX-C genes¹ and thus in *Pc* mutant embryos *Ubx* should be derepressed. However, *Ubx* protein products are not expressed at the high level found in parasegment 6 in the wild type. This is readily explained if the other genes of the BX-C are also expressed along the length of the animal in *Pc* embryos. The regulation of *Ubx* protein expression by *abd-A* and/or *Abd-B* (perhaps together with other homoeotic genes) then results in the observed ubiquitous moderate level of *Ubx* protein expression.

Our results support the model that the *Ubx* proteins represent the executive functions of the *Ubx* complementation group⁵, as the subfunction mutations all affect the distribution of *Ubx* products. The subfunction mutations appear to map outside

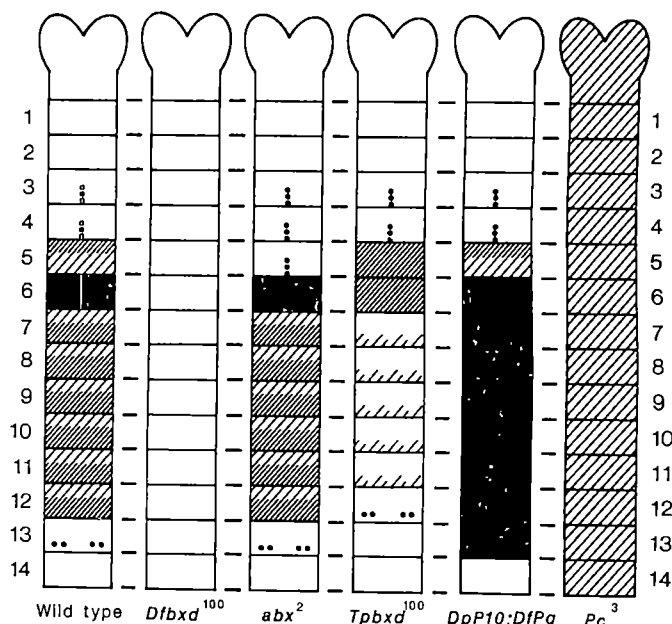
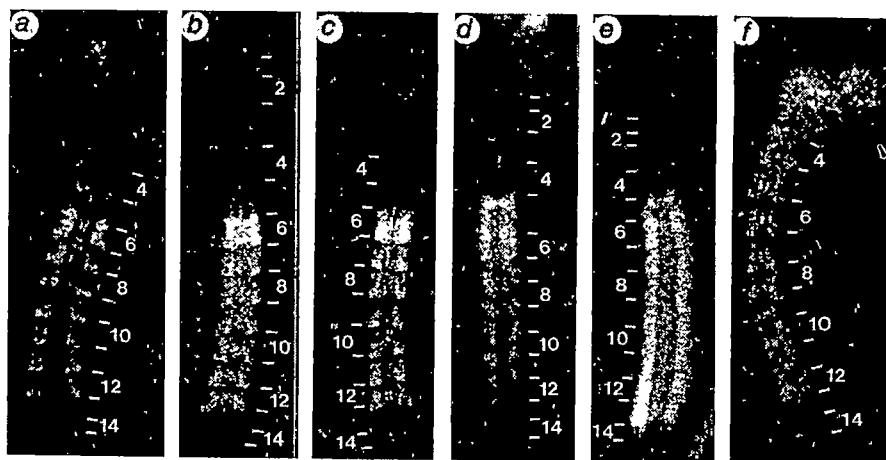


Fig. 5 Schematic diagram of *Ubx* protein expression in the VNS in wild-type and mutant embryos. The parasegmental units are indicated at the sides of the figure (see Fig. 4 for descriptions). *Dfbxd*¹⁰⁰ homozygous embryos lack the *Ubx* domain but contain the *abd-A* and *Abd-B* regions (see Fig. 1). They exhibit no labelling with FP.3.38 (ref. 16).

known coding sequences for *Ubx* products^{3,5} and behave as mutations in *cis*-regulatory sequences¹¹. However, it is not clear to what extent the specificity of their effects reflects the existence of specific wild-type regulatory functions rather than being a property of the mutations themselves. It has already been noted that the subfunction mutations differ in the nature of their DNA lesions as well as in their location³. For example, most *bx* mutations that have been examined are insertions of the *Gypsy* transposable element³ and developmentally regulated activity of the *Gypsy* promoter elements²³ may be important for their specific phenotypes.

Investigation of the patterns of *Ubx* transcript¹⁵ and protein¹⁷ distribution in wild-type embryos and larvae suggests a complex

programme of spatial and temporal control. Although we know little about the regulation of these patterns, we have established here that, in the stage-13 embryo, interactions between the genes of the BX-C have an important role. The key to understanding the subfunction effects may well lie in the interactions regulating the pattern of *Ubx* expression rather than in the individual *Ubx* products.

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Contrabithorax mutations cause inappropriate expression of Ultrabithorax products in *Drosophila*

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The mutation *Contrabithorax* (*Cbx*) has played an important part in the recognition of the role that genes of the bithorax complex (BX-C) play in the control of development. *Contrabithorax*, a dominant mutation which maps in the proximal or Ultrabithorax domain of the BX-C, results in the transformation of structures of the second thoracic segment (T2) into homologous structures of T3 (ref. 1). Recessive mutations and deletions of the same chromosome region have the reverse effect, transforming T3 into T2. This reciprocal relationship between the effects of dominant (gain of function) and recessive (loss of function) mutations suggested that products of the BX-C were both necessary and sufficient to specify the unique developmental pathways of different segments². Here we report that the original *Contrabithorax* mutation (*Cbx*¹) and two other dominant mutations in the Ultrabithorax domain, *Cbx*³ (ref. 3) and *Haltere mimic* (*Hm*)^{3,4}, all cause the inappropriate expression in T2 of products from the *Ultrabithorax* (*Ubx*) transcription unit. This strongly supports the suggestion that these products execute the functions of the proximal or Ultrabithorax domain of the BX-C⁵.

The DNA lesion in *Cbx*¹ is an insertion of ~17 kilobases (kb) into intron sequences of the *Ubx* transcription unit. The inserted sequences duplicate, in inverse orientation, a region normally located 27-44 kb upstream from the *Ubx* transcription start site^{5,6}. Two models have been proposed to explain the effects of this lesion on the development of cells in T2 (ref. 6). The first model suggests that the inserted DNA encodes a function that is required for the development of T3. The new location of this DNA fragment in the *Cbx*¹ mutation may then induce the inappropriate expression of this function in cells of T2, causing them to follow the T3 developmental pathway. The second model suggests that the *Cbx*¹ insertion interferes with the normal spatial regulation of the *Ubx* transcription unit itself, and that it is the inappropriate expression of *Ubx* products in T2 which changes the developmental pathway of these cells.

We have approached this question by examining the distribution of *Ubx* transcripts and proteins in the wing imaginal disks of larvae homozygous for the *Cbx*¹ mutation. In the wild type, there is little expression of *Ubx* in the wing disks (which will give rise to most of the dorsal structures of T2 in the adult), but *Ubx* transcripts and proteins are relatively abundant in the homologous disks of T3, which will give rise to the haltere, and in the T3 leg disks^{7,8}. In *Cbx*¹ homozygotes, *Ubx* transcripts and proteins are expressed in regions of the wing disk at levels comparable with those found in the normal T3 disks (Figs 1, 2).

The patterns of *Ubx* expression can best be seen in whole-mounts of wing disks which have been labelled for *Ubx* proteins (Fig. 2). Although details of the protein distribution vary from disk to disk, a high level of *Ubx* proteins is usually observed throughout most of the posterior compartment of *Cbx*¹ wing disks, with patches of expression also in the anterior compartment. This distribution correlates well with the cuticular phenotype seen in adults homozygous for the *Cbx*¹ mutation⁹. The posterior compartment of the second thoracic appendage is frequently transformed to T3, whereas the anterior compartment is transformed less frequently, and only in patches. This correspondence between cuticular phenotype and the expression of *Ubx* strongly suggests that the inappropriate expression of *Ubx* products is the cause of the observed transformations.

We have also examined the distribution of *Ubx* products in wing disks carrying the *Cbx*³ and *Hm* mutations. Both of these mutations have phenotypes analogous to that of *Cbx*¹, but the regions of T2 which are transformed to T3 are different for each of the three mutations³. *Cbx*³ results in a variable transformation which at its most extreme affects the entire anterior compartment of the wing and notum, but does not transform the posterior compartment (Fig. 3). *Ubx* proteins are also expressed in variable, but often extensive, regions in the anterior compartment of the *Cbx*³ wing disks, and the boundary of *Ubx* expression frequently defines a line at or close to the location of the anterior/posterior compartment boundary. The *Hm* mutation has a much more uniform phenotype: the entire wing blade is transformed to haltere tissue, but the notum is unaffected. In this case, a relatively constant pattern of *Ubx* expression is observed in the wing disk. Regions of the disk which will form the wing blade express high levels of *Ubx* proteins, but specific cells in other regions of the disk also express *Ubx* (Fig. 2).

None of these is simply a 'constitutive' mutation, resulting in the indiscriminate expression of the *Ubx* gene. In the case of *Cbx*¹ the only structures of the third-instar larva in which we have seen an effect of the mutation are the T2 dorsal and ventral disks. In other disks (for example, eye-antennal), and in other tissues, which do not normally express *Ubx* (for example, brain lobes, midgut), neither *Ubx* transcripts nor proteins are detectable (Fig. 1 and data not shown). Abnormally high levels of *Ubx* products are observed, however, in parasegment 5 of the embryonic nervous system (data not shown). In the case of *Hm* we have seen the inappropriate expression of *Ubx* in certain epidermal cells other than the wing disks, including cells of the anterior spiracle, but not in endoderm or most other tissues.

It is not yet clear how the effects of these dominant 'gain of function' mutations relate to the normal spatial regulation of

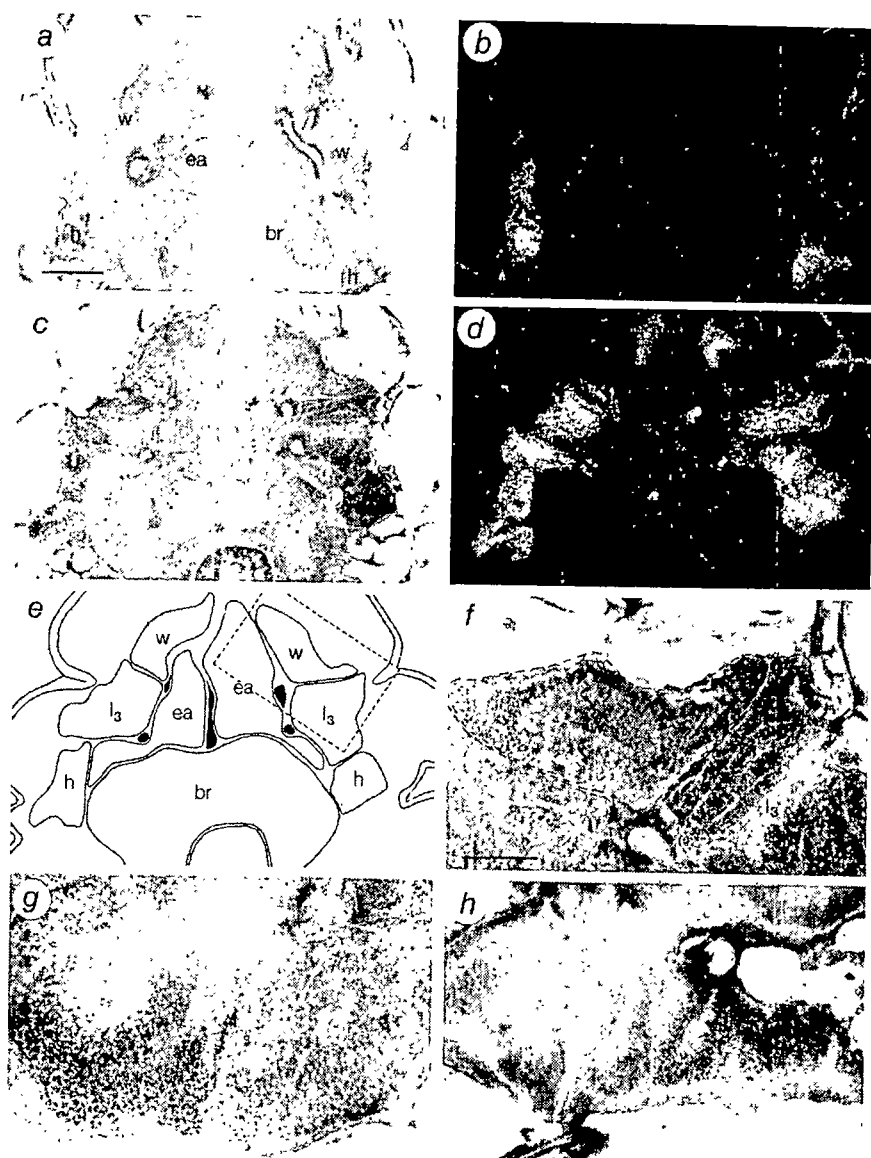
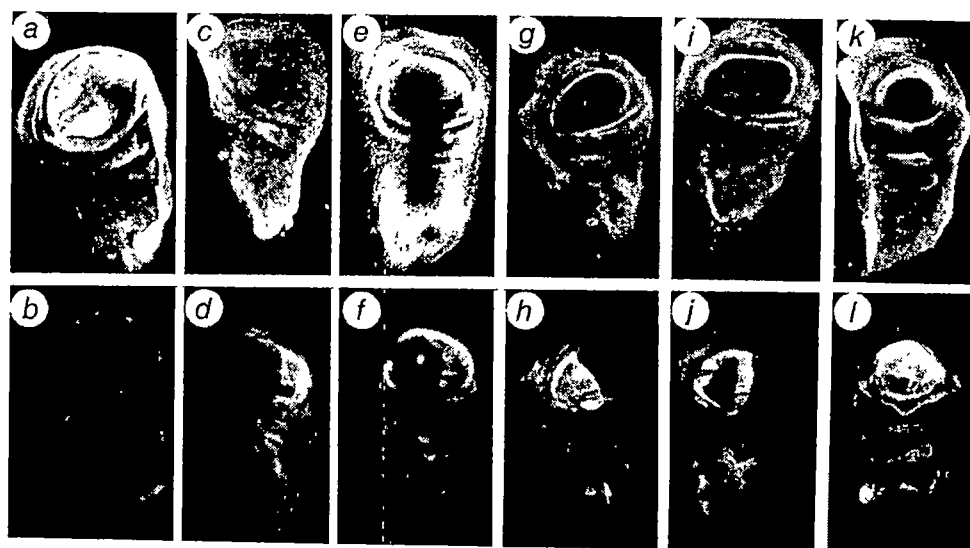


Fig. 1 Hybridization of *Ubx* probes to *Cbx* and *Hm* tissue sections. *a, b*, Bright- and dark-field photograph, respectively, of an autoradiograph of a horizontal section through the anterior of a wild-type third-instar larva. The *Ubx* probe hybridizes to the haltere disks (*h*) of T3, but not to the wing disks (*w*) of T2, nor to the eye-antennal (*ea*) disks or brain (*br*). (See ref. 7 for further details of the wild-type pattern.) *c, d*, Bright- and dark-field views of a comparable autoradiograph from a *Cbx*¹/*Cbx*¹ larva. The outlines of structures visible in this section are indicated in *e* and the boxed region is shown enlarged in *f*. The *Ubx* probe hybridizes to the disks of T3 (haltere and third leg (*l*₃), and also to regions of the wing disk, but not to eye-antennal disks or brain. *g*, Wing disk from *Cbx*¹/*Cbx*¹ third-instar larva, showing extensive transcription of *Ubx*. *h*, Wing disk from a *Hm*/+ third-instar larva, again showing a large region of *Ubx* transcription. Scale bars: *a-d*, 100 μ m; *f-h*, 50 μ m.

Methods. Probe preparation and hybridization conditions were as described in Fig. 3 legend of ref. 7. Note that the hybridization probe used is single-stranded (synthesized on the M13 template A113), and will therefore hybridize only to 5'-3' transcripts from the 5' exon of the *Ubx* transcription unit, and not to any transcripts originating from a promoter in the DNA of the *Cbx*¹ insertion, which would traverse the *Ubx* 5' exon in reverse orientation. Autoradiographic exposures were as follows: *a, b*, 15 days; *c-h*, 24 days.

Fig. 2 *Ubx* protein expression in wild-type and mutant wing imaginal disks. In the upper line the disks are labelled with the DNA-binding dye Hoechst 33258 to reveal the total pattern of nuclei. In the lower line the disks are labelled with the monoclonal antibody FP.3.38 to reveal the pattern of *Ubx* protein expression (see ref. 8 for details of this procedure). The disks are oriented with the anterior compartment on the left. *a, b*, Wild type. There is weak labelling on the posterior side of the disk, principally in the widely spaced nuclei of the peripodial membrane. *c-f*, *Cbx*¹/*Cbx*¹ disks. In *d* the labelling appears to fill the posterior compartment of the disk and the label boundary correlates well with the position of the anterior/posterior compartment boundary¹³. A more complex pattern is observed in the disk shown in *e* and *f*. It is not clear whether the entire posterior compartment is labelled, and there is patchy labelling in the anterior compartment. *g-j*, *Cbx*³/+ disks. In *h* the labelling appears to be restricted to the anterior compartment and, within this, principally in the presumptive distal region of the disk. The sharp boundary in the distal region probably coincides with the anterior/posterior compartment boundary. The disk in *j* shows an example of less extensive expression. The sharp boundaries of *Ubx* protein expression in *h* and *j* coincide with morphological discontinuities visible in the Hoechst-labelled disks (but barely discernible in these photographs, *g* and *i*). Thus, in *g* there is a single discontinuity corresponding to the single boundary of *Ubx* expression in *h*, and in *i* there are two discontinuities in the central distal area corresponding to the boundaries in *j*. Presumably, these discontinuities reflect cell-surface differences under *Ubx* control. *k, l*, *Hm*/+ disks: *Ubx* proteins are expressed in a complex pattern, mostly over the distal region of the disk. Other *Hm*/+ disks examined showed a very similar pattern of *Ubx* protein expression.



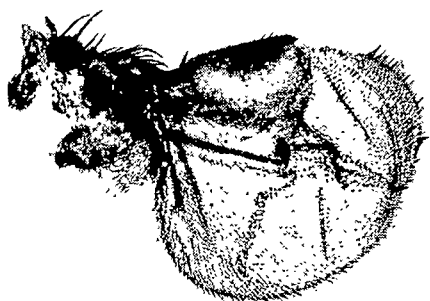


Fig. 3 The wing of a *Cbx*³/+ fly. Anterior is at the top and the proximal regions are to the left. The transformation of anterior structures is virtually complete in the distal regions, where the cuticle is covered with small, densely packed trichomes characteristic of the haltere. Proximally, the transformation is less complete, with many wing hinge structures remaining.

the *Ubx* gene. In the case of *Cbx*¹, there is reason to believe that the effect of the mutation on *Ubx* expression does reflect, in an aberrant way, the normal functions of the transposed DNA sequences. The *Cbx*¹ insertion arose together with a reciprocal deletion of *Ubx* upstream sequences, and was only subsequently separated by recombination¹⁰. Deletion of these sequences generates a *postbithorax* mutation¹⁰, which greatly reduces *Ubx* expression in the posterior part of the haltere disk¹¹. Thus, the deletion and relocation of these *Ubx* upstream sequences have virtually reciprocal effects on *Ubx* expression, suggesting that the transposed element normally has a role in the regulation of *Ubx*, principally in posterior compartments.

The *Cbx*³ mutation probably affects a different regulatory element. The only DNA lesion detected in this chromosome is an inversion break at the extreme 3' end of the *Ubx* transcription unit, 60 kb from the site of the *Cbx*¹ insertion⁶. This mutation allows the expression of *Ubx* in a region where it is normally never active, but has little or no effect on the expression of *Ubx* in those regions where it is normally active; flies homozygous for the *Cbx*³ mutation survive, and are virtually normal in T3 and A1 (M.E.A., unpublished observations). A second mutation with a very similar phenotype (*Cbx*^{nov}) proves to be an inversion with one break at virtually the same site in the BX-C⁶; this suggests that both mutations affect a regulatory element located near the 3' end of *Ubx* which is involved in the repression of *Ubx* in the region immediately anterior to its normal domain of expression.

The *Hm* mutation is associated with a chromosome rearrangement broken within 4 kb of the *Ubx* transcription start site¹², suggesting that it may result from the fusion of unrelated upstream elements to the *Ubx* gene.

These three dominant mutations, of very different structure, are alike only in that they result in the expression of *Ubx* proteins in the wing disk. It seems likely that any DNA rearrangement which has this effect, however fortuitously, will result in a 'Contrabithorax' phenotype.

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Distribution of *Ultrabithorax* proteins in mutants of *Drosophila bithorax* complex and its transregulatory genes

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The phenotypic effects of mutations have classically been described in terms of their penetrance, expressivity and specificity¹. These three parameters can give insights into the wild-type function of the genes affected by the mutations; for example, the topographic specificity of homeotic mutations provides a crucial clue to our understanding of the normal function of homeotic genes in development. The null alleles of such genes have constant specificity², and the expectation is that these genes are expressed in all the cells of the compartments affected by their mutations. However, hypomorphic mutations of the 'selector' genes that specify compartment identity are also known which have only partial, and sometimes variable, specificity. We have now studied the distribution of protein products of the *Drosophila Ultrabithorax* (*Ubx*) gene in various mutants, both of the *Ubx* domain of the bithorax complex and of *trans*-regulatory genes predicted to affect *Ubx* expression³. We find that the topographical specificity of the mutations correlates with the distribution of *Ubx* protein. In particular, in partially transformed compartments we find sharp within-compartment variation in the level of *Ubx* protein expression. This may correspond to alternative steady states of gene activity established by cell-cell interactions, which we suggest may reflect processes of normal development involved in embryonic compartmentalization.

The *Ubx* products were visualized by immunofluorescence using a monoclonal antibody specific for the protein products of the common 5' exon of the *Ubx* transcription unit⁴. The distribution of the *Ubx* proteins was studied in imaginal disks of mature larvae. Positive label only indicates the presence of at least one of the *Ubx* products and negative label indicates the absence of all or the most abundant proteins⁴. Fate maps of the imaginal disks considered here, the wing and haltere disks^{5,6}, allow us to correlate the adult transformation with the distribution of *Ubx* products in the mutant imaginal disks (Fig. 1a).

Previous studies using this antibody have shown that the *Ubx* proteins have a non-homogeneous distribution in the wild-type imaginal disks⁷, something not expected on the basis of mutational analyses. Thus, *Ubx* proteins appear in both compartments of the haltere disk, but show a more extreme label in the posterior one. Even within compartments, certain regions regularly show more label than others (Fig. 1c). *Ubx* label also appears in the pleural region of the posterior compartment of the wing disk⁴ (Fig. 1b). This is expected because *Ubx* mosaics show transformations in the posterior mesopleura^{8,9}.

The *Ubx* domain can be subdivided into two regions. One codes for RNAs that become translated into different proteins, the *Ubx* transcription unit, while the second is a *cis*-regulatory region coding for several RNAs, possibly involved in the differential splicing of the *Ubx* transcripts¹⁰; it is in this region that *postbithorax* (*pbx*) and *bithoraxoid* (*bxd*) mutations map¹¹.

We have analysed the distribution of *Ubx* products in several mutants of the *Ubx* region. One of them, *bx*^{34c}, shows, with variable expressivity but constant specificity, partial transformations to anterior wing and mesonotal structures in the dorsal metathorax⁸. This transformation is associated with an enlarged haltere in the presumptive wing territory (compare Fig. 1c with 1d). Figure 1d shows an example of the *Ubx* products in the transformed *bx*^{34c} haltere disks. The pattern of immunofluorescence varies slightly between imaginal disks of the same genotype. It holds for all cases observed, however, that distinct patches with only weak (and in some cases absent) label

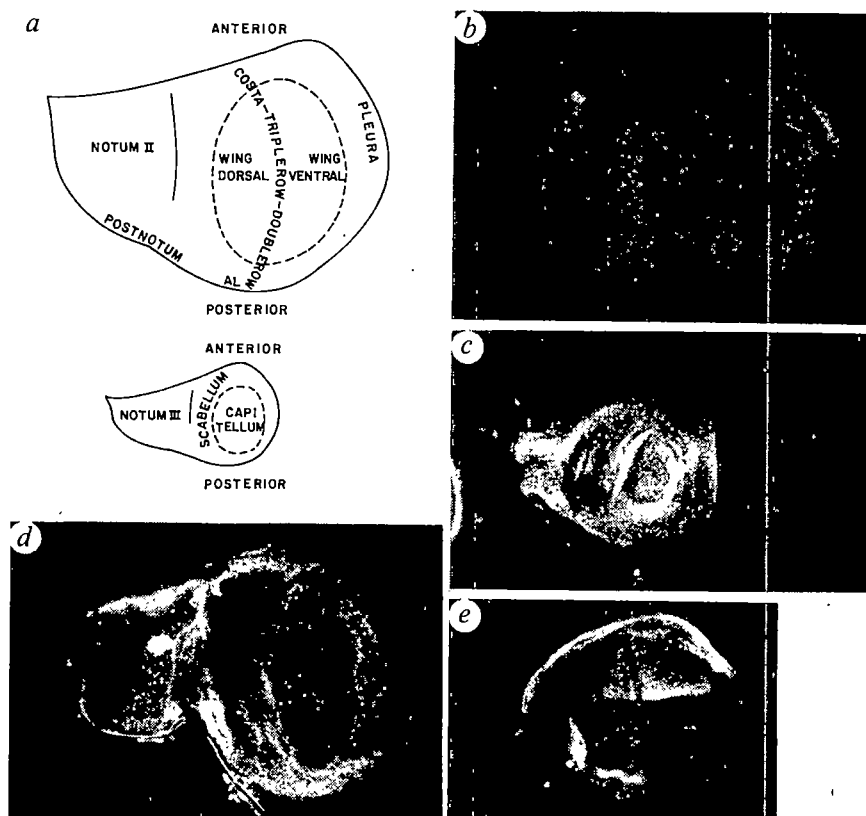
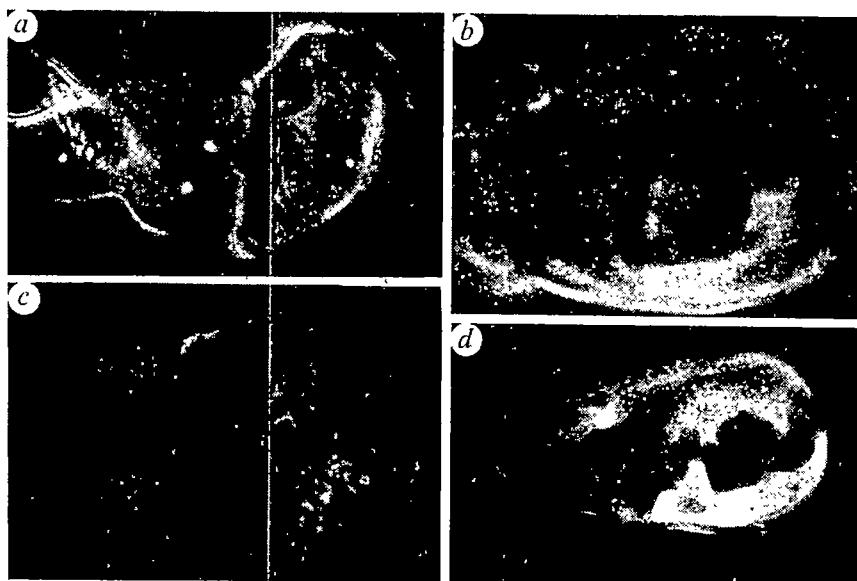


Fig. 1 Immunofluorescence patterns of *Ubx* expression. *a*, Fate maps of wing and haltere imaginal structures^{5,6}. Upper is anterior and lower posterior throughout. *b*, Wild-type wing disk; ventral view showing the posterior peripodial membrane (pleura) positively stained. *c*, Wild-type haltere disk. *d*, *bx*^{34e}/*Df*(3*R*)*Ubx*¹⁰⁹-transformed haltere disk with dark patches of unlabelled tissue in the anterior compartment. *e*, *pbx*¹/*Df*(3*R*)*Ubx*¹⁰⁹-transformed haltere disk with a large fraction of the posterior compartment showing negative staining. Note the positive label of the peripodial membrane.

Methods. Larvae were obtained from outcrosses of stocks balanced with *TM6B*, carrying a dominant marker (*Tubby*) to distinguish the genotypes to be studied. Third-instar larvae were dissected in TBS (50 mM Tris pH 7.4, 150 mM NaCl), disks mounted in polylysine-coated slides and further treated as described elsewhere⁶. The first antibody was FP3.38 ascites fluid used in a 1:200 dilution. The second antibody was goat anti-mouse IgG conjugated to fluorescein (Sigma) used at 1:50 dilution. Slides were mounted in glycerol-propylgallate-TBS and photographed with Ektachrome 400 ASA film in a Nikon microscope using filter set IF460-485. All major disks and salivary glands were studied from each mutant. Several individuals (4–10) in each case were dissected to ascertain the constance of the pattern. Controls of wild-type and *Df*(3*R*)*Ubx*¹⁰⁹/+ disks were run in parallel. The latter genotype consistently showed a slightly reduced intensity of staining.

Fig. 2 Immunofluorescence patterns of *Ubx* expression. *a*, *T*(2;3)*Hm*/+ transformed wing disk. The positive label in the wing blade corresponds with the adult transformation to capitulum, the spots of label observed on the presumptive notum are due to *Ubx*-positive cells of the trachea. *b*, *Cbx*¹/+ transformed wing disk. The positive label appears in discrete patches in the posterior wing region. *c*, *Pc*³*Dp*(3;3)*P5*/+ wing disk with spots of positive label in the posterior region. *Dp*(3;3)*P5* is a tandem duplication of the entire bithorax complex (BX-C). Extra copies of the BX-C enhance the *Cbx*-like phenotype produced by *Pc* mutations, but never show mutant phenotype in *Pc*⁺ flies. The black hole in the middle of the disk is due to an air bubble. *d*, *Df*(3*R*)*red*^{P52}/*Rg-bx*^{trxA} (*Rg-bx*^{trxA}) (M. P. Capdevila, unpublished) haltere disk with patches of negative staining in the anterior region.



(Fig. 1*d*) appear in regions of the anterior compartment, corresponding in position and extent to the wing-transformed territories of the adult dorsal metathorax. The boundaries between strong and weak (or absent) signal are very precise, although along them some cells with intermediate levels of label can also be seen. Cell lineage analysis of *bx*^{34e} mutant halteres shows that even small clones, generated late in development, can include both wing bristles and trichomes with haltere characteristics. The boundaries between these two types of tissue result from late developmental decisions in cells unrelated by lineage, that is, the transformation is not clonal (G. Morata and P. A. Lawrence personal communication, and unpublished observations of this laboratory).

The *pbx* mutants show, with variable expressivity and constant specificity, transformation of posterior haltere to posterior wing structures⁵. The *pbx*¹ haltere disks (Fig. 1*e*) show sharp boun-

daries between the putative transformed (enlarged) and non-transformed tissue. The distinct patches, which here are devoid of label, correspond in position and extent to the adult wing transformations (see also ref. 12). The absence of label observed in *pbx*¹ haltere disks supports the hypothesis that the *pbx*-*bx**d* region regulates *Ubx* in *cis* (refs 10, 13). We do not know whether the *pbx* transformations are of clonal origin.

Dominant mutations due to gain of function of the *Ubx* gene are generically designated *Contrabithorax* (*Cbx*). They are associated with molecular alterations in the DNA, within (*Cbx*¹) or near (*Hm*: *Haltere mimic*) the *Ubx* coding region (ref. 11 and W. Bender, personal communication). All the known *Cbx* mutants differ in specificity. We present here data for *Cbx*¹ and *Hm* (ref. 14 and Fig. 2). *Cbx*¹ heterozygotes show, with variable expressivity and constant specificity, a transformation of mainly the posterior wing into posterior haltere tissue, with smooth

boundaries. This transformation is not clonal¹⁵. *Hm* / +, on the other hand, presents distal (both anterior and posterior) compartments of the wing transformed into haltere tissue (capitulum). The histotypical borders are rather sharp, but the transformation is not clonal, at least from early stages of development (M. P. Capdevila, personal communication). The mutant wing disks of both *Cbx*¹ and *Hm* retain positive label in pleural regions but also have *Ubx* products in the regions that correspond to the transformations observed in the adult (Fig. 2a, b). In both cases, sharp boundaries can be observed between labelled and unlabelled cells. A pattern of label similar to that of wild type has been detected in the mesothoracic leg disk (labelled) and in the more anterior thoracic or cephalic eye-antennal disk (no label). The heterozygotes for these mutations show a normal pattern of *Ubx* products in both haltere and metathoracic legs.

The non-clonality of *bx* and *Cbx* transformations shows that they do not result from early segregation of lineages of cells. The adult mosaic transformations probably result from interactions between neighbouring cells during the development of the imaginal disks. The discontinuity of *Ubx* label observed in alleles causing either loss or gain of function corresponds to strong quantitative differences in *Ubx* products. These boundaries probably reflect discontinuity of territories with cells in either of two steady states—one typical of full expression of the *Ubx* gene, the other of lack of *Ubx* products. This reinforcement/extinction pattern of expression in neighbouring, genetically identical cells further suggests that regulatory mechanisms are involved in the maintenance of gene activity. Therefore, we studied the pattern of expression of *Ubx* products in flies mutant for two *trans*-regulatory genes of *Ubx*; these were *Polycomb* (*Pc*)^{16,17} and *Regulator of bithorax* (*Rg-bx*)^{18–20}.

Mutations in *Pc* cause a *Cbx*-like transformation in the wing of heterozygotes and mutations in *Rg-bx* cause *bx*-like transformations in the halteres of heterozygotes. The *Cbx*-like phenotype produced by *Pc* is of variable expressivity but of constant specificity; haltere tissue appears in the posterior wing border and gradually goes into wing tissue. This transformation is not clonal¹⁷. The *Ubx* label of *Pc*³ wing disks appears as multiple scattered spots in the presumptive region of the adult transformations, against a wild-type unlabelled background (Fig. 2c). The transformations caused by *Rg-bx* mutations are of variable penetrance, full expressivity but erratic specificity¹⁸. These transformations are not clonal, at least from early stages of development²⁰. The haltere disks of *Rg-bx* mutants show erratic patches of unlabelled tissue, separated by sharp boundaries from fully labelled territories (Fig. 2d). The patches appear restricted to the anterior compartments of haltere disks as are the transformations seen in the adult cuticle.

Genetic arguments suggest that both *Pc* and *Rg-bx* genes are involved in the regulation of *Ubx* expression both in embryogenesis and during imaginal cell proliferation^{3,19,21}. The genetic model suggests that *Pc*⁺ acts as a repressor and that *Rg-bx*⁺ has a positive role in the expression of *Ubx* (ref. 21 and J.B. and A.G.-B., unpublished data). The present results support these arguments and indicate further that these genes could be involved in the reinforcement/extinction process suggested above. Since both *Pc* and *Rg-bx* transformations are not clonal, the reinforcement/extinction process must extend to neighbouring cells. We do not know the polarity of these influences (expressing as opposed to non-expressing cells), neither do we know which are the genes of the syntagma involved in these interactions. Possibly these mechanisms also affect selector gene expression in regeneration and *trans*-determination²².

Our results indicate that cell interactions must be involved in determining the spatial patterns of expression during imaginal disk cell proliferation. In addition, we postulate that the same mechanisms also take place in early specification. *Ubx* transcripts appear in blastoderm stages, first as uniformly distributed over the presumptive mesothorax, metathorax and first abdominal segments, and later becoming restricted to posterior mesothorax, metathorax and anterior first abdominal²³. The

extinction in the mesothorax is not complete, but remains in the posterior pleural cells. We postulate that *Cbx* mutations affect this process of spatial restrictions, retaining in part the early embryonic pattern of transcription. Why this pattern is retained in an allele-specific manner in the different *Cbx*-like mutations is not known. The reinforcement/extinction process in mutants with partial loss of function is also reminiscent of the mechanisms involved in compartment specification. Early diffused patterns of transcription over several compartments become sharply delineated in the cells of wild-type individuals, as imaginal cells mutant for hypomorphic alleles do within compartments during proliferation. Again, we do not know why these patterns are allele-specific topographically. Indeed, pattern specificity, in both normal and mutant organisms, remains the most elusive problem in developmental genetics.

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Identification of the gene responsible for human T-cell leukaemia virus transcriptional regulation

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Human T-cell leukaemia viruses (HTLVs) have genomic organization distinct from that of other replication-competent retroviruses, possessing four genes, *gag*, *pol*, *env* and χ . The unique fourth gene, χ (also referred to as *lor*), is located between *env* and the 3' long terminal repeat (LTR), encoding a protein of relative molecular mass 40,000 for HTLV-I and 37,000 for HTLV-II^{1,2}, located in the nucleus of infected cells^{3,4}. HTLV-I is the causative agent of adult T-cell leukaemia (ATL), a T-lymphocyte malignancy^{5,6}, while HTLV-II has been found associated with a T-cell variant of hairy cell leukaemia^{7,8}. Both viruses immortalize T cells *in vitro*^{9–11}. However, the mechanism of cellular transformation induced by HTLV is not known as there seems to be no common site of provirus integration in primary ATL cells¹² and the virus contains no classical oncogene sequences^{13,14}. These observations have provoked speculation that the unique and strongly conserved χ protein (85% amino-acid homology between HTLV-I and -II) is involved in HTLV leukaemogenesis. Recent mutagenesis experiments in our laboratory have shown that the χ gene is essential

for HTLV replication¹⁵. It has also been shown that the LTRs of HTLV and the related bovine leukaemia virus (BLV) are activated *in trans* in virus-infected cells¹⁶⁻¹⁹, and, although such experiments did not directly demonstrate a role for the χ protein in transcriptional activation, it has been suggested that the χ protein is responsible for the transcriptional activation of the LTR and may be involved in cellular transformation^{3,4,16}. We have now developed a transient co-transfection assay which demonstrates that transcriptional activation of the HTLV LTR is mediated solely by the χ protein and that no other virus genes are required.

Others who have examined transcriptional regulation of the LTRs of HTLV or BLV have used constructions where transcrip-

tion from the LTR is measured by chloramphenicol acetyl transferase (CAT) activity following transfection of virus-infected cells¹⁶⁻¹⁹. We have used a highly sensitive transient co-transfection system to study activation of the LTR where selection of infected or transformed cells would not affect the results obtained. This system has the further advantage that the effects of individual virus genes can be isolated both from one another and from effects seen in immortalized virus-infected cells.

Figure 1 shows the schematic structure of the HTLV transcriptional units in pH6neo, cH6-H11, SV-HTLV and SV-3.9. In pH6neo and cH6-H11, both of which contain a complete provirus genome from the infectious HTLV-II clone λ H6 (ref. 20), transcription of HTLV-II genes would be expected to occur from the native cap site in the 5' LTR. In SV-HTLV, the HTLV insert is an 8.2-kilobase (kb) *Bam*HI fragment of the virus which extends from the 5' LTR to the 3' LTR but lacks the 5' 361 nucleotides (U3 and part of the R region) and 3' 462 nucleotides (part of R and U5) of the HTLV-II genome. The viral genes are expressed using simian virus 40 (SV40) transcription sequences present in the vector. Removal of the HTLV promoter region from the U3 portion of the 5' LTR and HTLV termination sequences from the 3' LTR results in transcription being promoted by the upstream SV40 promoter-enhancer region and terminated in the SV40 termination-polyadenylation sequences downstream of the HTLV insert. SV-3.9 has a 3.9-kb fragment of HTLV-I which extends from genomic position 4,990 in the *pol* gene to near the 3' end of the provirus genome and encodes all of the sequences necessary for χ gene expression²¹. Transcription is promoted by the upstream SV40 promoter-enhancer region of the vector, as is the case with SV-HTLV, but terminates in the 3' LTR rather than in the SV40 sequences of the vector (Fig. 1).

Figure 2a shows the results of co-transfections in COS cells with each of these constructions and either HTLV-I LTR-CAT (LTR-I-CAT) or HTLV-II LTR-CAT (LTR-II-CAT)^{15,22}. Transcriptional activation of the LTR-CAT constructions occurs

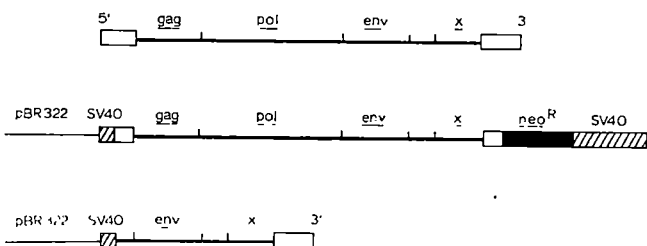
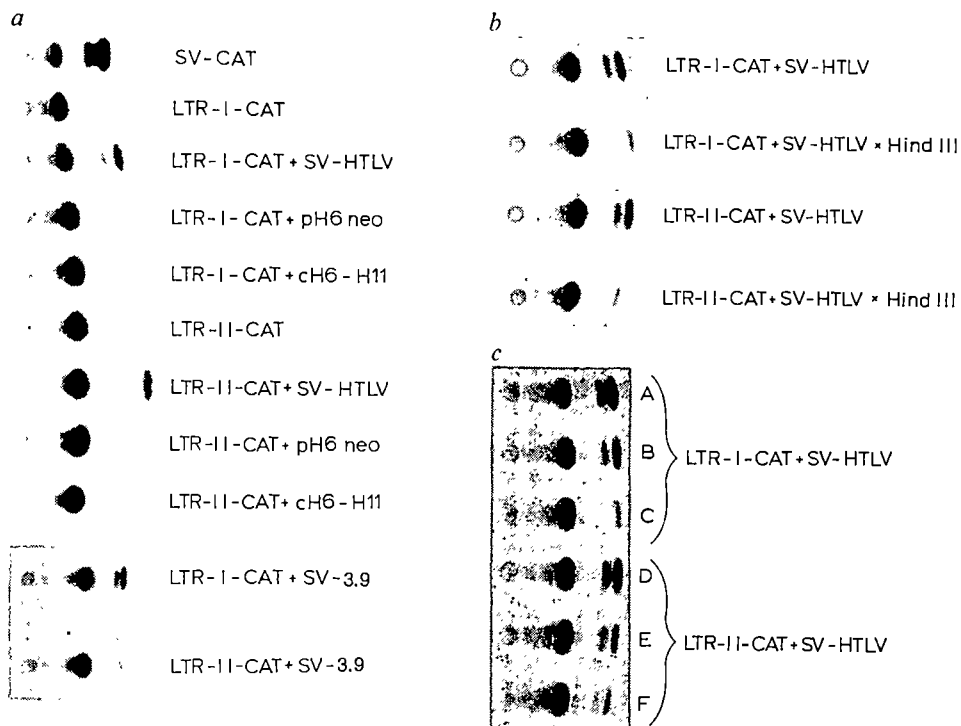


Fig. 1 Schematic structure of constructions used in transfection experiments. Upper line: HTLV-II insert of pH6-neo in the vector pSV2-neo (ref. 34) and cH6-H11 in the vector pSVod (ref. 35). The entire HTLV-II genome (8.9 kb) plus some flanking cellular DNA is present in both constructions. Middle line: structure of construct SV-HTLV, which has an 8.2-kb HTLV-II insert (nucleotide positions 362-8,550) in vector pSV2-neo. The 8.2-kb fragment from a partial *Bam*HI digestion was treated with the large fragment of *Escherichia coli* DNA polymerase I. *Hind*III linkers were added and the fragment was ligated to *Hind*III-digested pSV2-neo. Lower line: structure of construct SV-3.9. A 3.9-kb fragment from the 3' end of the HTLV-I genome (from a *Hind*III site at nucleotide 4,990 to an *Eco*RI site created by *Bal*31 nuclease digestion of HTLV-I proviral DNA and insertion of a synthetic oligonucleotide at position ~8,890) was ligated into pSV2-neo doubly digested with *Hind*III and *Eco*RI. The neomycin resistance and SV40 termination-polyadenylation sequences of pSV2-neo were deleted, and transcription of the HTLV-I insert is terminated in the 3' LTR.

Fig. 2 CAT activity measured in COS cells transfected with HTLV constructions. **a**, High levels of CAT are expressed from a modified construct of the SV40-based vector pSV-CAT²⁶ on transfection into COS cells. The structure of the HTLV-I LTR-CAT construction (LTR-I-CAT) and HTLV-II LTR-CAT (LTR-II-CAT) is as described elsewhere^{15,20}. Transfection with LTR-I-CAT or LTR-II-CAT alone produces a very low background CAT activity. Co-transfection with SV-HTLV produces CAT activity approaching that obtained with pSV-CAT. Co-transfection with constructions pH6-neo or cH6-H11 produces CAT activity not significantly higher than that obtained with LTR-CAT alone. Relative conversion of chloramphenicol into acetylated forms (expressed as a percentage of SV-CAT control) from at least three experiments was as follows: LTR-I-CAT, <1%; LTR-II-CAT, <1%; SV-HTLV+LTR-I-CAT, 16%; SV-HTLV+LTR-II-CAT, 9%; SV-3.9+LTR-I-CAT, 10%; SV-3.9+LTR-II-CAT, <1%. Less than threefold variation in levels of CAT activity observed between assays were not considered significant. **b**, *Hind*III digestion of SV-HTLV cleaves the construct at the ends of the HTLV-II insert. Co-transfection of LTR-CAT with *Hind*III-digested SV-HTLV abolishes (<1% SV-CAT control) the CAT activity seen in co-transfections with the native DNA, indicating that both the SV40 and HTLV-II sequences present in the construction are required to achieve *trans*-activation of LTR-CAT. **c**, Transfection of LTR-I-CAT (5 μ g) with 3.0 μ g, 0.6 μ g and 0.12 μ g of SV-HTLV (lanes A-C); LTR-II-CAT (5 μ g) with 3.0 μ g, 0.6 μ g and 0.12 μ g (lanes D-F) of SV-HTLV. pSV2-neo DNA was used as carrier to bring the total amount of DNA to 10 μ g in each case. Transfection with 0 μ g of SV-HTLV induces only a background level of CAT activity (**a**). Dose response to the HTLV-II vector SV-HTLV is similar for both LTR-I-CAT and LTR-II-CAT (see text). **Methods**. 2×10^6 COS cells were seeded on 100-mm plates and transfected after 24 h with 10 μ g of DNA, using the calcium phosphate co-precipitation technique³⁷. Cells were collected 40-48 h after transfection and CAT activity determined by TLC. The amount of CAT activity is indicated by conversion of ¹⁴C-radiolabelled chloramphenicol (left) into acetylated forms (right). Results were quantified by cutting out bands and determining the amount of radiolabel in each by liquid scintillation counting.



only in co-transfections with the SV-HTLV and SV-3.9 constructs, and not in transfections with the other HTLV constructs. This is due to more efficient transcription of HTLV genes from the SV40 promoter than by the HTLV-II LTR (ref. 20 and see Fig. 2a). The HTLV-II construction SV-HTLV is capable of activating transcription from both the LTR-I-CAT and LTR-II-CAT constructs, but the HTLV-I construct SV-3.9 efficiently activates only LTR-I-CAT. This is consistent with the observations of other investigators who found that the LTR of HTLV-I is activated in HTLV-II-infected cells but that the HTLV-II LTR is only weakly active in HTLV-I-infected cells¹⁶. *Trans*-activation is abolished in co-transfections with *Hind*III-digested SV-HTLV, in which the HTLV-II insert is removed from the SV40 promoter/termination sequences in the vector (Fig. 2b). Figure 2c shows the dose-response obtained by co-transfection of COS cells with LTR-CAT and decreasing amounts of the SV-HTLV construction. Efficient expression of the CAT gene by the LTR is clearly dependent on the presence of the intact SV-HTLV construct in the transfection; dilution or restriction endonuclease digestion of SV-HTLV decreases or abolishes the activation of LTR function. Therefore, transcriptional activation of the LTR is dependent on expression of HTLV functions which are supplied in these co-transfection experiments by SV-HTLV. Similar results were obtained in co-transfections of other cell lines, including HeLa cells and lymphoid cells such as Epstein-Barr virus-transformed B cells (data not shown). We will refer

to this *trans*-acting transcriptional activation of LTR functions as the *trans*-regulatory function of HTLV.

To determine which of the HTLV genes was responsible for activation of the LTR, we introduced mutations into the SV-HTLV construction described above. These mutants were used in the COS cell co-transfection system together with LTR-CAT (see Fig. 3). The HTLV χ gene is encoded by a 2.1-kb messenger RNA derived by the splicing of two introns, the first of which contains the *gag* and *pol* genes and the other the major part of the *env* gene and the untranslated region of the genome²¹. The middle exon supplies four nucleotides which encode the NH₂-terminus of the χ protein. These four nucleotides are derived from the *env* gene methionine codon and one additional nucleotide which is spliced to the major χ open reading frame of the third exon. Only mutations which affect the expression of χ (SV-HTLV-Xho and SV-HTLV-Cla) (see Fig. 3 legend for details of constructs) abolish transcriptional activation. Other mutations in the *gag*, *pol* and *env* genes (SV-HTLV-Sst, Xba, EXB) did not abolish the *trans*-regulatory function of the genome. Note that mutants bearing deletions in the 700-bp nontranslated region of the HTLV-II genome (SV-HTLV-ExB

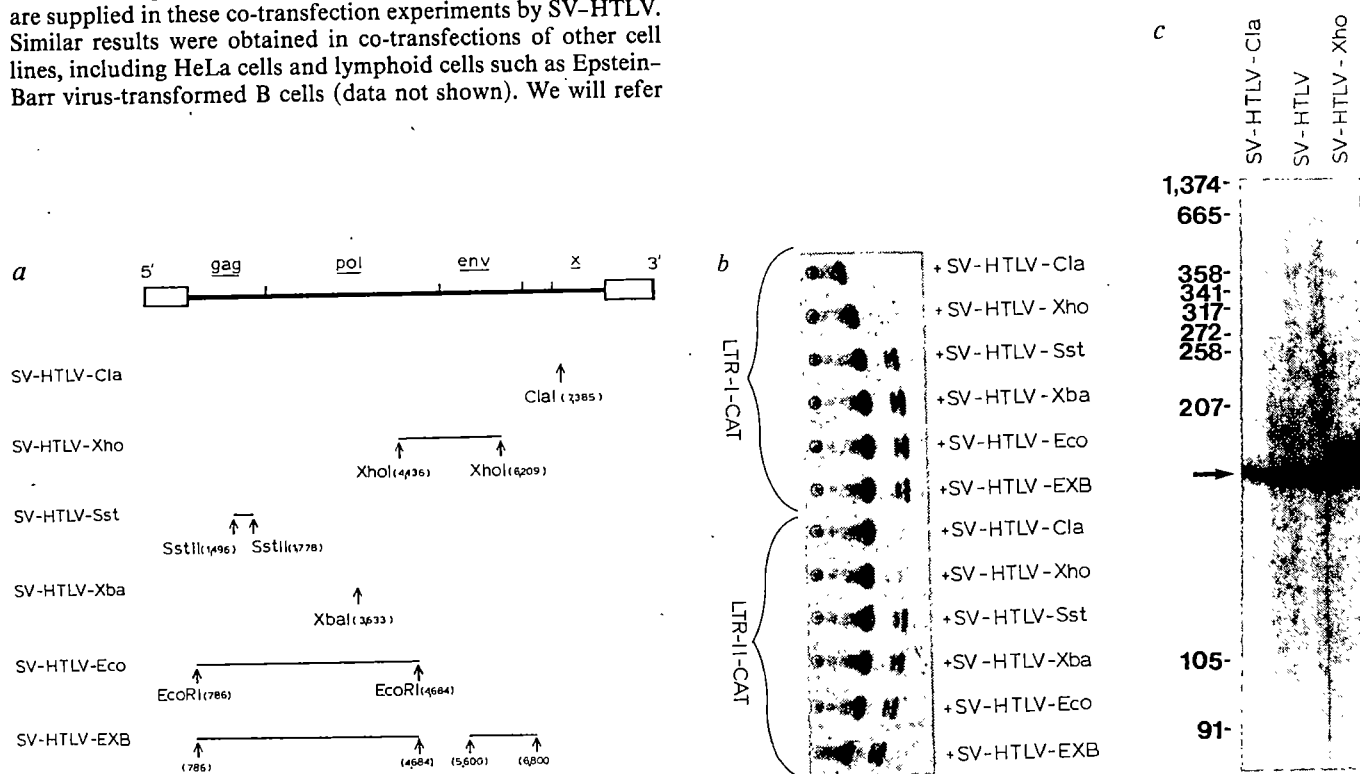


Fig. 3 a, Location of mutations introduced into SV-HTLV. The mutant SV-HTLV-Sst has a 309-bp deletion in *gag* p24 (position 1,469–1,778) and activates the LTR in co-transfection assays. SV-HTLV-Xba has a frameshift mutation in the *pol* gene, due to a 4-bp insertion at position 3,633. These four bases were introduced by treatment of *Xba*I-digested SV-HTLV with the large fragment of *E. coli* DNA polymerase I and recircularization with DNA ligase. Although this mutant would be expected to produce a truncated *pol* gene product, this does not affect *trans*-regulation of the LTR. SV-HTLV-Eco has a 3,898-bp deletion (788–4,686), created by removal of an *Eco*RI fragment, which deletes the whole of the *gag* region and most of the *pol* gene but leaves intact *env* and χ gene coding sequences²¹. SV-HTLV-EXB was derived from SV-HTLV-Eco by a *Bal*31 nuclease deletion at a *Xho*I site at position 6,209 in the *env* gene of SV-HTLV-Eco. The *Bal*31-induced deletion in this mutant is ~1.2 kb long and extends from the *env* glycoprotein gp52 at the 5' end into the nontranslated region upstream of the χ open reading frame (position ~5,700–6,900). This mutant has deletions of *gag*, *pol* and *env* which total 5.1 kb of the 8.2-kb HTLV-II insert in SV-HTLV and is still capable of *trans*-activating both LTR-CAT constructions efficiently. SV-HTLV-Xho contains a 1,773-bp deletion (4,436–6,209) in the HTLV insert which deletes the 3' portion of the *pol* gene, *env* glycoprotein gp52 and the 5' portion of the transmembrane protein p20. Although this mutant retains the major coding region of the χ gene in the third exon, it is not expected to produce a functional χ gene product because of the deletion of the middle exon containing the initiation codon required for χ gene expression²¹. Co-transfections with this mutant do not produce increased CAT activity over that seen with LTR-CAT alone. SV-HTLV-Cla has a frameshift mutation in the χ gene, due to a 2-bp insertion into *Cla*I-digested SV-HTLV at position 7,385. This change is expected to produce an altered χ protein. The native p37^{HTLV-II} protein (relative molecular mass 40,000) comprises a total of 339 amino acids, 337 of which are coded between nucleotide positions 7,190 and 8,202 in HTLV-II. The altered predicted protein from SV-HTLV-Cla comprises 158 amino acids, that is, 67 from the native protein at the N-terminus and 91 amino acids from a different reading frame at the C-terminus (position 7,660). This predicted protein, unlike the native protein produced by SV-HTLV, cannot activate LTR-CAT. b, CAT activity in COS cells co-transfected with LTR-CAT and SV-HTLV mutants. Cells were transfected and CAT activity measured as described for Fig. 2. Lanes 1–6 received 5 μ g of LTR-I-CAT; lanes 7–12, 5 μ g of LTR-II-CAT. 5 μ g of DNA from each mutant were used as indicated on the figure. Relative conversion of chloramphenicol into acetylated forms (as a percentage of SV-CAT control) from at least three experiments was as follows: LTR-I-CAT+SV-HTLV-Cla, <1; +SV-HTLV-Xho, <1; +SV-HTLV-Sst, 20; +SV-HTLV-Xba, 21; +SV-HTLV-Eco, 10; +SV-HTLV-EXB, 15; LTR-II-CAT+SV-HTLV-Cla, <1; +SV-HTLV-Xho, <1; +SV-HTLV-Sst, 17; +SV-HTLV-Xba, 22; +SV-HTLV-Eco, 11; +SV-HTLV-EXB, 13. Less than threefold variation in levels of CAT activity observed between assays was not considered significant. c, S₁ nuclease analysis of 50 μ g of cytoplasmic RNA from transfected COS cells. S₁ analysis of χ mRNA was performed using a *Hha*I/*Cla*I ³²P-5' end-labelled probe (nucleotide positions 6,957–7,387) for the HTLV-II χ splice-acceptor site (position 7,214). Processed χ mRNA is indicated by the presence of a protected DNA fragment of 174 nucleotides, indicated by an arrow (see ref. 38).

and others; data not shown) between *env* and χ also retained *trans*-regulatory function, indicating that this region is not involved in expression of *trans*-regulatory functions as assayed by co-transfection.

S₁ nuclease analysis was used to examine the production of χ RNA by the SV-HTLV mutants. An S₁ nuclease probe specific for processed χ mRNA demonstrated that comparable levels of mRNA were produced by each of the constructions including the χ gene mutant SV-HTLV-Cla, indicating that the defect in SV-HTLV-Cla is in the translation of a functional χ gene product, not in χ mRNA levels (Fig. 3c). The apparently aberrant splicing observed with SV-HTLV-Xho may be due to loss of the second exon in the normal χ mRNA. These results demonstrate that only mutations which interfere with correct processing (SV-HTLV-Xho) or translation (SV-HTLV-Cla) of χ gene mRNA prevent *trans*-regulation of LTR-CAT. Other mutations which do not prevent expression of χ , including those that abolish all other viral genes, *gag*, *pol* and *env* (SV-HTLV-EXB), do not affect LTR *trans*-regulation, indicating that expression of the χ protein appears to be the single factor mediating *trans*-regulation and that no other virus gene is required.

HTLV differs from other retroviruses by the presence of a distinct locus, the χ gene, which is responsible for transcriptional regulation. A transcriptional activator locus has also recently been identified in a different reading frame of the *gag* gene of Rous sarcoma virus²³. There appears to be no comparable locus in HTLV, as mutants which completely lack the *gag* gene still activate the LTR efficiently. Unlike the χ gene of HTLV¹⁵, it is not known whether the *gag* activator locus is essential for replication of Rous sarcoma virus. These studies show directly that the requirement of the χ gene for viral replication is due to the transcriptional regulatory functions of the χ gene on the LTR. The presence of the χ gene may provide additional advantages for transcriptional regulation of HTLV expression in the host cell. The HTLV-II construct SV-HTLV can activate both the HTLV-I LTR and HTLV-II LTR, indicating a similar function for the χ proteins of both viruses, consistent with the sequence homology between the HTLV χ proteins^{13,14} and similar biological properties of HTLV-I and -II χ proteins⁵⁻⁸. The failure of the HTLV-I construct SV-3:9 to activate transcription from LTR-II-CAT is probably due to differences in the LTRs of HTLV-I and -II.

The function of the χ gene in HTLV transcription and replica-

tion must have a major influence on cellular transformation by, and on the pathogenicity of, HTLV. The kinetics of HTLV replication following infection may be related to the level of χ gene expression. Following integration into the genome of the host cell, low levels of χ mRNA are transcribed. Production of small amounts of the χ protein would stimulate LTR function, in turn producing higher levels of χ protein, and so on. This hypothesis is consistent with the slow kinetics observed in HTLV infection of cells (J.D.R., unpublished) and contrasts with typical retrovirus infections, where stable levels of RNA expression merely require passage of the infected cell through the S phase of the cell cycle^{24,25}.

Functional analogies exist between the HTLV χ gene and transcriptional regulatory genes of some DNA viruses. The best studied example of such a gene is the *E1a* gene of adenoviruses. Like χ , the product of *E1a* regulates adenovirus transcription by stimulating the transcription of other adenovirus genes^{26,27}. Since adenoviruses are capable of transforming certain cell types and *E1a* itself is required to transform cells^{28,29}, it seems likely that the HTLV χ gene may also be involved in cellular transformation. Structural and functional similarities have recently been found between the cellular oncogenes *myc*, *myb* and adenovirus *E1a* proteins³⁰, and we have recently shown that there are striking functional similarities between χ and *E1a*³¹. It is becoming apparent that the χ gene shares functional similarities with other transcriptional regulatory proteins, and like them, may also be involved in oncogenesis, possibly by causing aberrant transcriptional regulation in cells in which they are expressed (see refs 32, 33). Studies on the similarities between χ and other proteins known to be capable of cellular transformation will help to determine the role of the χ protein itself and the influence of altered transcriptional regulation as a mechanism for carcinogenesis.

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Note added in proof: Since the submission of this manuscript, two additional reports have confirmed the role of the χ gene in transcriptional activation of the HTLV LTR^{39,40}.

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Dissociation of transforming and *trans*-activation functions for bovine papillomavirus type 1

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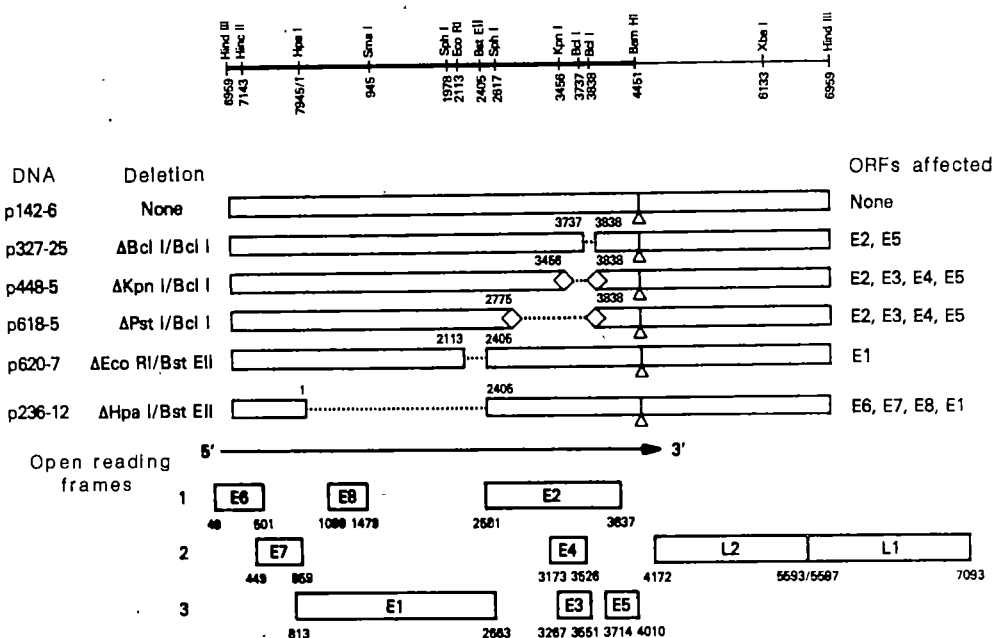
It has been shown that genetic information encoded by the 3' open reading frames (ORFs), E2, E3, E4 and E5, of bovine papillomavirus type 1 (BPV-1), is sufficient to induce cellular transformation of certain mouse cells¹⁻⁵. The product of the E2 ORF has further been shown to be responsible for the *trans*-activation of a transcriptional regulatory element located in the noncoding region (NCR) of the BPV-1 genome⁶. To examine whether or not the E2 *trans*-activation function is encoded by the same gene that encodes the 3' ORF viral transformation function, we have now analysed the expression of the *trans*-activation function in series of mouse C127 cells transformed by BPV-1 deletion mutants. In addition, using mutated complementary DNA clones generated by the insertion of a premature translational termination linker into different sites of a BPV-1 cDNA clone containing the 3' ORFs intact, we demonstrate that transformation and transcriptional *trans*-activation functions can be dissociated and that they map respectively to the E5 and E2 ORFs.

BPV-1 or its cloned full-length DNA can transform certain rodent cells *in vitro* and the viral DNA persists as an extra-chromosomal multi-copy plasmid in these transformed cells⁷. The genetic organization of BPV-1 has been established from the analysis of the DNA sequence⁸ and the viral RNA transcripts (Fig. 1)^{3,9-13}. Mutagenesis studies have identified several regions of the BPV-1 genome which influence the expression of the viral transformation functions. Mutations affecting the E2 ORF result in a reduced ability to transform mouse cells, suggesting a role for the putative E2 viral gene product in the transformation process^{2,4,5}. Mutations affecting the E5 ORF also have a marked effect on transformation efficiency^{5,14-16}. The E6 ORF encodes

an independent transforming protein^{3,21} and its function is critical for expression of the fully transformed phenotype². A transcriptional enhancer, which is activated in *trans* by the putative E2 gene product, has been mapped within the 1-kilobase (kb) NCR of the BPV-1 genome⁶.

To examine whether or not the *trans*-activation function of the E2 gene product is linked to the BPV-1 transforming functions of the 3' ORFs, cell lines transformed by different BPV-1 deletion mutants (shown in Fig. 1) were tested for their ability to activate the NCR enhancer. Table 1 lists the cell lines assayed. C127 cells are not transformed and do not contain BPV-1 DNA sequences, and as such serve as a negative control. NS142-6 cells are C127 cells transformed by a plasmid containing the entire BPV-1 genome cloned into the pML2d vector and provide the factor capable of *trans*-activating the NCR enhancer present in p407-1 (ref. 6). The NS620-7 and NS236-12 cell lines have been transformed with BPV-1 deletion mutants that affect the 5' ORFs (E5, E7, E1 and E8). As previously reported, both of these cell lines can provide the *trans*-acting factor to activate p407-1, confirming that the 3' ORFs encode the diffusible *trans*-acting factor⁶. The NS327-25, NS448-5 and NS618-5 cell lines have each been transformed by BPV-1 mutants containing deletions extending various distances upstream from the *Bcl*I site at base 3,838. The p327-25 mutant has a 101-base deletion which affects the carboxy terminus of E2 as well as the 5' terminus of the E5 ORF, upstream of the first E5 AUG methionine codon. This deletion mutant has a transforming activity similar to that of full-length BPV-1 DNA. The mutants p448-5 and p618-5 contain larger deletions which affect the E3 and E4 ORFs as well as the E2 and E5 ORFs. These mutants transform at a level approximately 10% and 1%, respectively, of that of wild-type BPV-1 DNA or of p327-25 DNA². Consistent with previous studies mapping the *trans*-activating factor to the E2 ORF, none of these mutants were able to activate the NCR enhancer in p407-1, despite their varied transformation capability⁶. These data suggest that BPV-1-mediated transformation and a transcriptional activation imparted by the E2 ORF gene product can be unlinked. It is of interest that the activity of the BPV-1 NCR chloramphenicol acetyl transferase (CAT) plasmid p407-1 is higher in those lines transformed by BPV-1 plasmids mutated in the E1 ORF than in the line transformed by the BPV-1

Fig. 1 Structure of the BPV-1 DNA deletion mutants used in transforming mouse C127 cells assessed for providing the NCR/CAT *trans*-acting factor. The recombinant plasmid and deletion are indicated on the left. Each of these plasmids is cloned in pML2d at the *Bam*HI site as indicated by an open triangle. The retained sequences (open box) are covalently linked at the sites indicated and in the figure are separated by the deleted sequences (...). *Xho*I linkers are indicated by a diamond. The ORFs for the BPV-1 genome are indicated at the top of the figure, as are the bases defining the beginning and the ends of each of the ORFs. The E6, E7, E1 and E8 ORFs are referred to in the text as the 5' ORFs because of their location, and the E2, E3, E4 and E5 ORFs are referred to as the 3' ORFs. No significantly large ORFs are located between the *Hind*III site (base 6,959) and the *Hpa*I site (base 7,945/1) and this segment is referred to as the noncoding region (NCR). The construction of each of these deletion mutants has been described previously². The ORFs affected by the deletion mutants are listed on the right. These constructions are presented relative to the map of BPV-1 ORFs and the linear genome. All of the detectable polyadenylated mRNA species are transcribed from a single strand and all ORFs are located on the same strand.



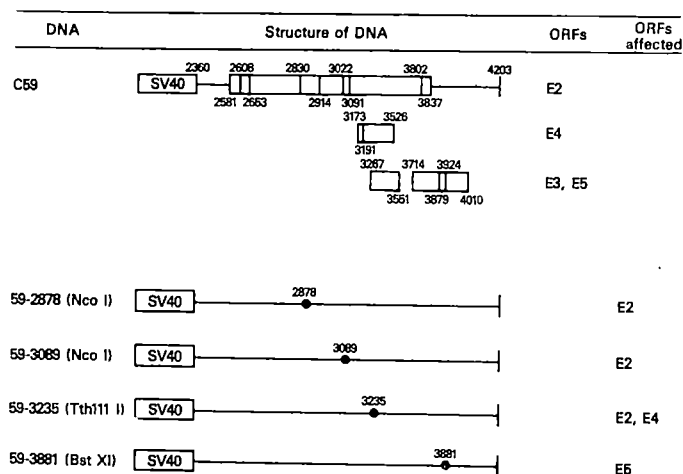


Fig. 2 Structure of the C59 cDNA clone and derived mutants. The structure of this clone is shown at the top of the figure with the SV40 early promoter and late introns located 5' to the BPV-1 ORFs³. The E2, E3, E4 and E5 ORFs are contained intact in the C59 clone. Each of these ORFs is indicated by an open box. Vertical lines in the open boxes represent the AUG codons which could potentially be used to initiate translation. Numbers adjacent to the ORFs represent the 5' and 3' boundaries of the ORFs as well as the location of the AUG codons. The mutants containing the linker fragment with the premature termination codon are indicated below with the position of the in-frame termination codon indicated. The sequence of the synthetic premature translation termination linker which also contains a restriction endonuclease site for *Hpa*I is

TAAGTTAACTAG
ATTCAGTTCAT

ORFs which would be affected by the insertion of the premature termination codon linker are listed on the right.

full-length genome. Whether this is simply due to experimental cell line variability or to a negative transcriptional regulatory effect mediated by the product(s) of one or more of the 5' ORFs is under investigation.

To separate further the *trans*-activation and transformation functions within the BPV-1 genome, we have introduced a premature translational termination codon linker at various positions into the BPV-1 cDNA clone, C59 (Fig. 2)³. This DNA can transform mouse C127 cells and *trans*-activate the BPV-1 NCR enhancer element^{3,6}. The C59 clone can be predicted to direct the synthesis of the putative E2 protein (relative molecular mass 48,000) with initiation and termination codons at nucleotides 2,608–2,610 and 3,838–3,840, respectively. This cDNA clone, however, also contains the E3, E4 and E5 ORFs intact. By inserting a synthetic linker containing translational stop codons in each of the three reading frames into the different ORFs, we were able to assess the role of each ORF in transformation and *trans*-activation. The structure of each of the resultant linker insertion mutants was verified by DNA sequence analysis and each was shown to contain a single linker insertion (data not shown). Each of the resultant mutants was assayed for its ability to transform mouse C127 cells and to *trans*-activate the NCR transcriptional regulatory element in p407-1 using an acute assay on monkey CV-1 cells (Table 2). The mutants C59-2878

and C59-3089, which affect only the E2 ORF, were unable to provide the diffusible factor for *trans*-activation of the BPV-1/ncr/CAT in the CAT assay; however, they retained the full transforming function on mouse C127 cells. The mutation in 59-3235, located at the *Tth*III site, affects the E2 and E4 ORFs and resulted in the loss of the *trans*-activation function but did not affect the transforming function. Only in 59-3881, in which the premature termination codon was inserted at the *Bst*XI site at base 3,881, was the transformation function abolished. The NCR *trans*-activation function, however, remained intact. The termination linker in 59-3881 is located in the E5 ORF downstream from the first AUG at base 3,879; the E2, E3 and E4 ORFs are intact in this mutant. The retention of *trans*-activation function in 59-3881 indicates that this mutation does not affect the stability of the E2 messenger RNA and strongly suggests that the E5 ORF has a critical role in transformation.

The role of the E5 ORF in transformation has been implicated in other studies using mutants possessing deletions or insertions in the full-length BPV-1 genome^{5,14,16} and in a subgenomic fragment of the BPV-1 genome promoted from a retrovirus long terminal repeat¹⁵. However, these initial studies have not revealed whether the mutations at the *Bst*XI site were altering transformation by affecting critical coding sequences or through regulatory sequences required in *cis*. As yet, no viral RNAs have been found splicing into the E5 ORF and no obvious splice acceptor sequence is located in the vicinity. The fact that the non-transforming mutant 59-3881 provides the *trans*-activation function argues that the mutation at the *Bst*XI site does not affect the stability or translatability of the viral RNA. These results, therefore, provide compelling evidence that the E5 ORF is a coding domain for a critical transforming protein. The E5

Table 1 Chloramphenicol acetyltransferase expression from CAT recombinant plasmids in C127 cells transformed by BPV-1 deletion mutants

Cell line	BPV-1 deletion in transforming DNA	ORFs affected	CAT activity		
			pA ₁₀ CAT	pSV2CAT	p407-1
C127	—	—	0.7	37.5	1.6
NS142-6 1	None	None	0.4	36.5	6.7
NS620-7 B	2,113–2,405	E1	0.6	26.7	17.5
NS236-12 B	1–2,405	E6, E7, E8, E1	0.1	11.2	12.7
NS236-12 C	1–2,405	E6, E7, E8, E1	0.2	42.2	29.2
NS327-25 A	3,737–3,838	E2, E5	0.6	33.7	1.4
NS448-5 A	3,456–3,838	E2, E3, E4, E5	1.1	26.2	3.1
NS618-5 B	2,775–3,838	E3, E3, E4, E5	1.7	29.7	3.3

Each of the cell lines was selected by its transformed phenotype. In each of the cell lines transformed by a BPV-1 deletion mutant, the BPV-1 DNA was in an integrated form². Each cell line was independently transfected with the plasmids pA₁₀CAT²⁶, pSV2CAT²⁷ and p407-1⁶ and assayed 48 h post-transfection for CAT activity. The pSV2CAT plasmid contains the *cat* gene in a modified eukaryotic transcriptional cassette promoted by the full simian virus 40 (SV40) early region promoter including the repeated 72-base-pair functional enhancer²⁷. The pA₁₀CAT plasmid was derived from pSV2CAT and lacks the functional enhancer but still contains the early promoter²⁶. The p407-1 plasmid was derived from pA₁₀CAT by the insertion of the BPV-1 *Hind*III/*Hpa*I NCR fragment in the *Bgl*II site of pA₁₀CAT, 5' to the SV40 early promoter in the transcriptional sense orientation⁶. CAT activity is measured as the % of chloramphenicol which is acetylated by the cellular extract in 30 min at 37 °C. The cellular extract in each assay was normalized for protein content before incubation. Deletions are given in terms of the nucleotide number of the BPV-1 genome.

Table 2 Transformation and *trans*-activation by cDNA clones containing termination codons

Clone no.	Transformation (foci per plate)	CAT activity		
		pA ₁₀ CAT	pSV2CAT	p407-1
C59	26, 40	0.4	61.0	61.0
C59-2878*	21, 31	0.3	55.0	1.7
C59-3089	23, 50	ND	ND	0.3
C59-3235	12, 42	0.3	70.8	1.2
C59-3881	0, 0	0.2	61.2	62.9
Salmon sperm DNA	0, 0	0.3	10.3	1.2

Transformation of C127 cells was carried out with 5 µg of the indicated cDNA clone using salmon sperm DNA as carrier as previously described³. CAT activity is measured as the % of chloramphenicol which is acetylated by the cellular extract in 30 min at 37 °C. CV-1 cells were transfected with each of the above cDNA clones listed and with the plasmids pA₁₀CAT²⁶, pSV2CAT²⁷ and p407-1⁶. Cell extracts were prepared 48 h post-transfection. ND, not determined.

* This clone has been referred to previously as C59N-9 (ref. 6).

ORF would be predicted to encode a 44-amino-acid hydrophobic protein.

The role of the product of the E5 ORF in the pathogenesis of a fibropapilloma is not known, although it may be involved in the proliferation of the dermal fibroblasts characteristic of a fibropapilloma. ORFs analogous to E5 in BPV-1 have been identified in other papillomavirus genomes that have been sequenced. The sequence of E5 ORFs of HPV-1a (ref. 17) and Shope papillomavirus¹⁸, which induce purely epithelial lesions, however, are not homologous to the E5 ORF of BPV-1. HPV-6b is associated with condyloma acuminata in humans and contains an E5a ORF which shares only slight homology with the E5 ORF of BPV-1¹⁹. There is, however, a small ORF in the corresponding region of the deer papillomavirus genome²⁰ and of the BPV-2 genome (W. D. Lancaster, personal communication) which has the potential to encode a peptide strikingly similar in size and amino-acid composition to the BPV-1 E5 ORF. Like BPV-1, the deer papillomavirus and BPV-2 each induces fibropapillomas in their natural host and fibroblastic tumours in hamsters, and readily transforms rodent cells in culture.

The mechanism by which the putative E2 gene product can *trans*-activate the NCR transcriptional regulatory element and its interaction with other viral or cellular genes during the transformation process remains unknown. The dissociation of the *trans*-activation and transformation functions of the 3' ORFs demonstrated here suggests that the E2 gene product may function indirectly through the activation of the NCR transcriptional regulatory element. The activation of the NCR enhancer by the E2 gene product could lead to increased transcription from the upstream promoter and expression of the 5' ORFs including E6, E6/E7 and E1, which have been shown to affect transformation and plasmid maintenance²⁻⁴. However, we have not ruled out the possibility that the E2 ORF gene product may have a direct effect on transformation. Our assay for transformation has been done using the mouse C127 cell line, which is an established cell line. It is possible that the E2 ORF has a direct transformation function but one that must be assayed in a different cell system. Other viral and cellular genes whose products have *trans*-activation properties, including the adenovirus E1a gene²² and the *c-myc* gene²³, have been shown to interact with other transforming genes in transforming primary cells^{24,25}. It is possible that *trans*-activation is only one of several pleiotropic functions of the E2 gene product.

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Forensic application of DNA 'fingerprints'

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Many highly polymorphic minisatellite loci can be detected simultaneously in the human genome by hybridization to probes consisting of tandem repeats of the 'core' sequence¹. The resulting DNA fingerprints produced by Southern blot hybridization are comprised of multiple hypervariable DNA fragments, show somatic and germline stability and are completely specific to an individual^{2,3}. We now show that this technique can be used for forensic purposes; DNA of high relative molecular mass (M_r) can be isolated from 4-yr-old bloodstains and semen stains made on cotton cloth and digested to produce DNA fingerprints suitable for individual identification. Further, sperm nuclei can be separated from vaginal cellular debris, obtained from semen-contaminated vaginal swabs, enabling positive identification of the male donor/suspect. It is envisaged that DNA fingerprinting will revolutionize forensic biology particularly with regard to the identification of rape suspects.

Biological samples for forensic analysis consist mainly of bloodstains or semen stains on cloth or other surfaces (often several days or even weeks old), vaginal swabs taken after an alleged rape, and sometimes hair roots^{4,5}. Current methods for typing blood and semen stains are reviewed by Divall⁶ and Sensabaugh⁷. Typically, a battery of polymorphic protein and blood group marker systems are used (the Metropolitan Police Forensic Science Laboratory, London, currently use ABO, adenylate deaminase (ADA), adenylate kinase (AK), carbonic anhydrase (CA-II), erythrocyte acid phosphatase (EAP), esterase D (EsD), glyoxalase I (GLO), haemoglobin (Hb), peptidase A (Pep A), phosphoglucomutase (PGM₁), Gm, Lewis and rhesus (Rh) for blood analysis). Detection of PGM₁, EAP, ADA and Rh is difficult after 26 weeks^{7,8}; EsD and CAII cannot be reliably detected after 1 month⁷. In contrast, only five systems are at present in common use for identifying genetic markers in semen (ABO, PGM, GLO, Pep A and Lewis) and all must be used with considerable caution because semen samples analysed are often contaminated with vaginal fluid which itself has enzyme and blood group activity. Semen contains proteolytic enzymes which further reduce the amounts of proteins recovered⁹. Furthermore, bacterial activity may be responsible for producing erroneous results, particularly in the identification of ABO blood groups^{10,11}.

DNA fingerprints can only be obtained from high- M_r undegraded DNA. Previous attempts to isolate DNA from dead or aged material have been reported from 140-yr-old quagga muscle¹² and 2,400-yr-old mummies¹³. In both cases only low- M_r DNA was obtained, and it was therefore necessary to establish whether intact DNA could be isolated from samples which may be typically encountered in forensic laboratories.

Multiple samples were prepared from 11 different donors. Bloodstains and semen stains were prepared by aliquoting fresh material onto clean cloth which was air-dried before storage at ambient temperature and humidity. Hair roots were analysed

Fig. 1 Isolation of high relative molecular mass DNA from dried bloodstains, semen stains and hair roots. Lane 1, fresh blood (60 μ l); lane 2, 6-week-old bloodstain (60 μ l equivalent); lane 3, 20-month-old bloodstain (60 μ l equivalent); lane 4, fresh semen (20 μ l); lane 5, 5-week-old semen stain (20 μ l equivalent); lane 6, degraded sample of 5-yr-old semen stain (40 μ l equivalent); lane 7, fresh hair roots ($\times 15$) (RNA is also clearly visible from hair roots); lane 8, fresh blood (60 μ l); lanes 9–14, control human placental DNA, 0.1 μ g, 0.2 μ g, 0.4 μ g, 0.8 μ g, 1.6 μ g, 3.2 μ g respectively.

Methods. Whole blood, semen, hair roots, bloodstains and semen stains were incubated overnight in 1.5-ml microcentrifuge tubes at 37 °C in 0.01 M Tris-HCl, 0.01 M EDTA, 0.1 M NaCl (pH 8.0) containing 2% SDS, 20 μ g ml⁻¹ proteinase K and 0.039 M DTT. DNA was purified by two phenol/chloroform extractions and precipitated by the addition of 0.1 volume of 2 M sodium acetate and 2.5 volumes of absolute ethanol. The DNA was pelleted by centrifugation at 15,000g for 5 min, washed with 70% ethanol and repelleted. If a residue remained (suggesting contamination by low- M_r compounds), the sample was dissolved in 20 μ l of sterile distilled water and passed down a small spun column (200 μ l Sephadex G-50 contained in a 0.75-ml microcentrifuge tube)¹⁵. Samples were electrophoresed on a 10-cm 0.5% agarose gel at 80 V for 2 h, and DNA was visualized by staining with ethidium bromide.

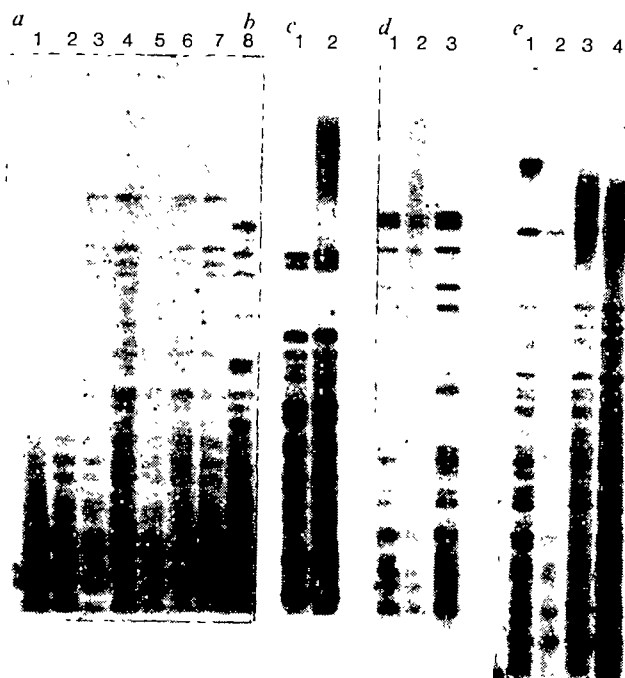
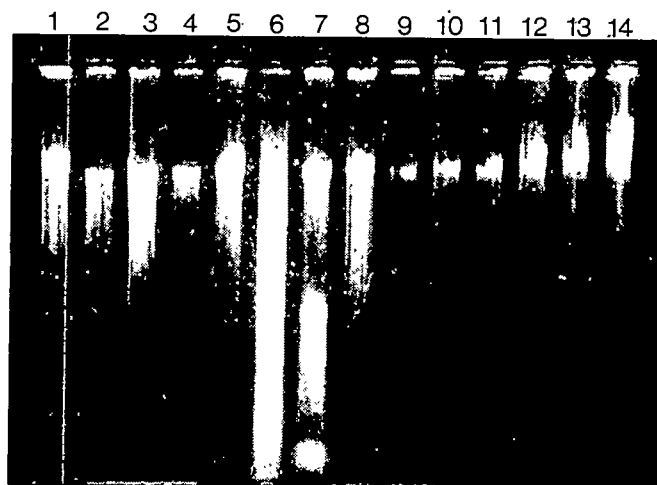


Fig. 2 Somatic stability and ageing studies. Individual *a*: lane 1, degraded sample from 5-yr-old semen stain (40 μ l equivalent); lane 2, 4-week-old semen stain (40 μ l equivalent); lane 3, fresh semen (40 μ l); lane 4, whole blood (60 μ l); lane 5, 4-week-old bloodstain (60 μ l equivalent); lane 6, 2-yr-old bloodstain; lane 7, fresh hair roots ($\times 15$). Individual *b* (showing differences between DNA fingerprints of *a* and an unrelated individual on the same Southern blot): lane 8, fresh blood (60 μ l). Individual *c*: lane 1, fresh semen (20 μ l); lane 2, 11-week-old semen stain (20 μ l equivalent). Individual *d*: lane 1, fresh semen (20 μ l); lane 2, 11-week-old semen stain (20 μ l equivalent); lane 3, hair roots ($\times 13$). Individual *e*: lane 1, fresh blood (60 μ l) (the largest band is high- M_r DNA at the electrophoretic exclusion point); lane 2, 1-day-old bloodstain (80 μ l equivalent); lane 3, 30-month-old bloodstain (80 μ l equivalent); lane 4, 4-year-old bloodstain (80 μ l equivalent).

Methods. Samples were prepared as described for Fig. 1. DNA was digested with *Hinf*I (20 units), in the presence of 4 mM spermidine trichloride, for 2 h at 37 °C. Samples were phenol extracted and ethanol precipitated before electrophoresis through a 20-cm long 0.6% agarose gel at 35 V for 20 h. DNA was denatured *in situ*, transferred to a Schleicher and Schull membrane filter (BA85) and filter-hybridized to ³²P-labelled single-stranded minisatellite probe 33.15 as described elsewhere^{1,2}. Autoradiography was carried out at -80 °C, with an intensifying screen, for 7 days.

fresh. Semen-free and semen-contaminated vaginal swabs (taken at a known period of time after intercourse) were also obtained together with blood samples from both the female and the male. All swabs were stored at -20 °C before use. DNA was extracted from whole blood, whole semen, vaginal fluid, hair roots, bloodstains and semen stains by overnight incubation in an SDS/proteinase K/dithiothreitol (DTT) mixture. Because preliminary experiments showed that semen-contaminated vaginal swabs contained large amounts of DNA from the female, tending to obscure many of the bands from the sperm, female cells were preferentially lysed by preliminary incubation in an SDS/proteinase K mixture. Sperm nuclei are impervious to this treatment because they are ramified with cross-linked thiol-rich proteins¹⁴ and can therefore be separated from the female component by centrifugation. Sperm nuclei were subsequently lysed by treatment with an SDS/proteinase K/DTT mixture. Electrophoresis of samples on 0.5% agarose gels showed that high- M_r DNA could be isolated from bloodstains and semen stains that were at least 2 years old and from fresh hair roots (Fig. 1). However, no DNA could be isolated from hair shafts.

DNA fingerprints obtained from all bloodstains, semen stains and hair roots were shown to be specific to individuals when compared with whole blood and semen samples (Fig. 2). Approximately 5 μ l of semen or equivalent semen stain and 60 μ l of blood or equivalent bloodstain were required. Some samples analysed were partial digests (Fig. 2e) but these also showed patterns consistent with those of a particular individual. DNA fingerprints were obtained from bloodstains up to 4 yr old (Fig. 2e). High- M_r DNA from a 5-yr-old semen stain (Fig. 1, lane 6; Fig. 2a, lane 1) and a 3-yr-old blood stain had degraded so that no pattern was visualized. After using the differential lysis technique only sperm DNA was isolated from a vaginal swab taken 6.5 h after intercourse (Fig. 3, lane 1).

These preliminary results demonstrate that DNA fingerprints are capable of changing completely the emphasis of blood-grouping in forensic science. Using eight polymorphic protein systems together, Sensabaugh⁷ quoted a probability of 0.014 for individual specificity. In practice, the degree of characterization is often much lower, particularly when semen is grouped. This leaves a large degree of uncertainty. In contrast, the condition



Fig. 3 DNA fingerprint from two vaginal swabs taken 6.5 h after intercourse (lane 1). The pattern is the same as that obtained from the male donor's blood (lane 2). The female's DNA fingerprint (from blood) is shown in lane 3.

Methods. Vaginal cells from semen-contaminated swabs were preferentially lysed by a preliminary incubation in the absence of DTT for 30 min using the lysis mixture described in Fig. 1 legend. Sperm nuclei were pelleted by centrifugation at 15,000g for 5 min, washed once, repelleted and lysed with the full SDS/proteinase K/DTT mixture. DNA was isolated as described in Fig. 1 legend and DNA fingerprints prepared (Fig. 2 legend).

of non-association has been absolute using traditional blood-grouping tests, that is, if the phenotype does not match, a common origin is not possible. Using two DNA minisatellite probes (33.15 and 33.6)¹, the degree of association can approach certainty—thus the probability of chance association using probe 33.15 is $<3 \times 10^{-11}$, and if two probes are used (33.15 and 33.6) the probability is substantially reduced to $<5 \times 10^{-19}$ (ref. 2). In short, using this single method, it is now possible for the forensic scientist to be positive about blood-grouping tests, whereas in the past, it was only possible to be sure of negative associations.

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Light regulation of plant gene expression by an upstream enhancer-like element

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Light regulates many varied physiological and developmental phenomena during plant growth and differentiation, including the formation of a photosynthetically competent chloroplast from a proplastid¹. The expression of ribulose 1,5-bisphosphate carboxylase small subunit (*rbcS*) genes is regulated by light in a development- and tissue-specific manner^{2,3}. In some plant species, phytochrome has been demonstrated to mediate this response⁴⁻⁷, and photoregulation of *rbcS* expression occurs at least in part at the level of transcription^{8,9}. We have shown previously that a 5'-noncoding fragment (4-973 base pairs (bp) upstream of the messenger RNA cap site) of the pea *rbcS* *ss3.6* gene contains all of the nucleotide sequence information necessary to direct the photoregulated expression of a bacterial chloramphenicol acetyltransferase (*cat*) gene in tobacco¹⁰. Consistent with these findings, Morelli *et al.*¹¹ have shown by deletion analysis of a second *rbcS* gene promoter, that the sequences required for photoregulated expression of *rbcS* E9 reside within the 5'-noncoding region. They identified an upstream region of ~700 bp needed for maximum transcription but not light-dark regulation, and a region from -35 to -2 bp which included the TATA box and contained the necessary information for light responsiveness. We now demonstrate that regulatory sequences 5' distal to the *rbcS* *ss3.6* TATA box and transcriptional start site not only contain the information necessary for maximum expression, but also confer photoregulation. These upstream regulatory sequences function independently of orientation when fused to their homologous promoter or a heterologous promoter.

The expression of many animal viral and cellular genes is influenced by regulatory sequences, commonly referred to as enhancers¹²⁻¹⁸, which characteristically function independently of orientation at various distances from the TATA box and start site of transcription^{12,16-18}. Certain enhancers activate gene expression only in specific cell types¹⁶⁻¹⁸, or in response to physiological stimuli¹⁵. It has not previously been investigated whether the expression of some plant genes is also regulated by enhancers.

The nucleotide sequence requirement for photoregulated expression of the *rbcS* *ss3.6* gene¹⁹ was examined using the CAT expression system²⁰. We and others^{10,20-23} have demonstrated the ability of this system to compare promoter efficiency and have shown that for chimaeric genes such as those used here, in which the mRNAs are identical, CAT activity reflects the cellular *cat* mRNA levels¹⁰ and therefore the transcriptional activity directed by the targeted promoter. To overcome any bias due to different RNA processing or translation efficiencies, the various test promoter fusions were always compared with the wild-type promoter.

To define more accurately the sequences within the -973 to -4 *rbcS* *ss3.6* promoter which are necessary to direct photoregulated expression, a series of promoter deletion mutants was constructed and tested for their ability to direct photoregulated *cat* gene expression in transformed tobacco calli (Fig. 1). Three of the constructs contain 5' deletions with endpoints at 722, 357 and 92 bp upstream from the cap site and the fourth contains a 3' deletion lacking sequences -4 to -90 (including the TATA box).

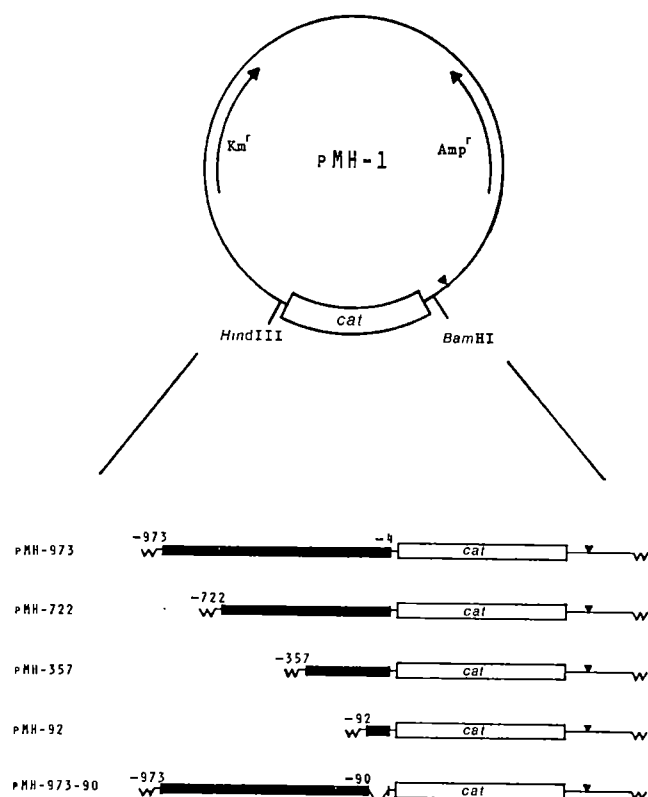
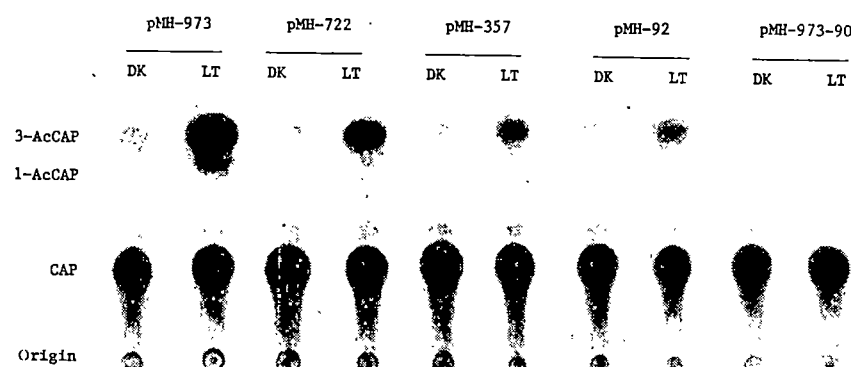


Fig. 2 Chloramphenicol acetyltransferase activity in transformed tobacco calli expressing truncated *ss-cat* chimaeric genes. Chimaeric genes containing truncated *ss3.6* promoters were introduced into tobacco cells and transformed calli grown in light or darkness. The upper panel shows a representative autoradiograph of thin layer chromatographic separation of CAT assay reaction products; the lower panel presents a summary of the data derived from these assays. The plasmid designation for the various deletion mutants appears above each pair of lanes. Extracts from light- and dark-grown tissues are designated LT and DK, respectively. CAP, ^{14}C -chloramphenicol; 1-AcCAP, 1-acetylchloramphenicol; 3-AcCAP, 3-acetylchloramphenicol.

Methods. Plasmids containing truncated *ss-cat* chimaeric genes were mobilized into *Agrobacterium tumefaciens* harbouring either the wild-type Ti plasmid, pTiC58, or the attenuated plasmid, pLGV-3851 (ref. 30), and stable recombinants were used to infect *Nicotiana tabacum* var. SR1 cells¹⁰. Several weeks after inoculation, tumours were removed from the plantlets and axenic homogeneous cell lines of transformed calli selected by hormone-independent growth. Independently derived transformants were subcultured and maintained at 28 °C both in darkness on MS media³¹ containing 3% sucrose and in light (12-h dark/light cycle) on MS media containing 0.1% sucrose and 0.2 $\mu\text{g ml}^{-1}$ 6-benzylaminopurine. Southern hybridization analysis of total DNA from transformed calli was performed to confirm the presence and integrity of introduced chimaeric genes (data not shown). CAT activity was assayed in extracts from transformed calli as described previously¹⁰, except that the reaction mixtures were preincubated at 37 °C for 5 min before the addition of acetyl CoA and the final concentration of acetyl CoA in the reaction was adjusted to 0.3 mM. Reaction products were separated in silica gel plates²¹ and located by autoradiography. Individual reaction products were cut from the silica plate and the radioactivity present determined by liquid scintillation. The amount of ^{14}C -chloramphenicol and its 1- and 3-acetyl derivatives were determined and activity is expressed as % conversion of chloramphenicol into its acetyl derivatives by 100 mg of tissue extract in 30 min. Induction is the ratio of CAT activity in light- versus dark-grown callus as determined from the per cent conversion. To calculate relative activity, levels of induction were compared with those of the -973 *rbcs ss3.6* promoter. The average of at least two to five experiments involving independently derived transformants is presented. The values presented are derived from experiments with cells transformed with chimaeric genes introduced using the *Agrobacterium* vector pGV3851.

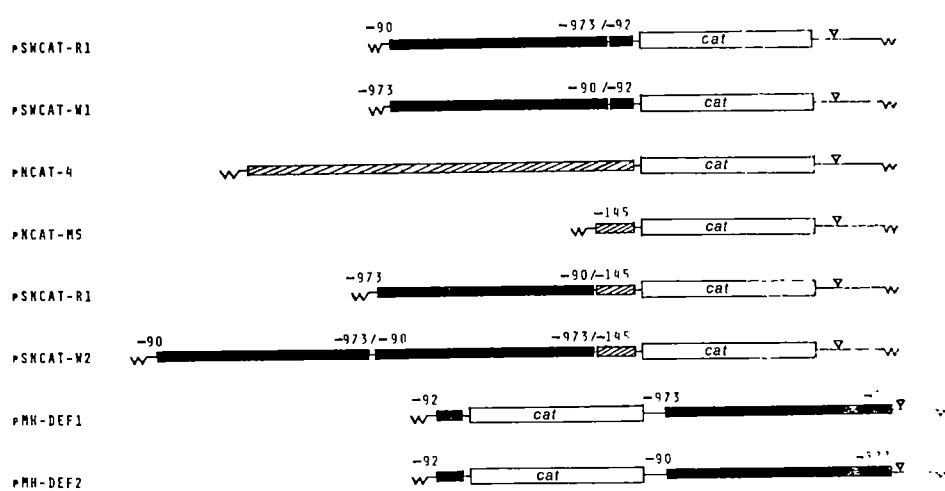
Fig. 1 The construction of *ss-cat* chimaeric genes under the control of truncated pea *rbcs ss3.6* promoter fragments. Plasmids encoding truncated *ss-cat* genes are designated by the endpoints of the truncated promoter fragment. Plasmid pMH-973 is equivalent to plasmid pMH-2 (ref. 10). Amp^r , ampicillin resistance; Km^r , kanamycin resistance; \square , chloramphenicol acetyltransferase (*cat*) coding sequences; ∇ , nopaline synthase (*nos*) polyadenylation signal; \blacksquare , *rbcs ss3.6* promoter sequences.

Methods. An ~1-kb *Hind*III restriction fragment containing nucleotide sequences extending from 4 to 973 bp 5' to the mRNA cap site of the pea *ss3.6* gene and 30 bp of pBR327 was isolated from plasmid pPSR6¹⁹ and cloned into the *Hind*III site of pSVO-cat²⁰. To generate 5' deletions, the resulting plasmid was linearized by restriction with *Nde*I and digested with *Bal*31 exonuclease. The ends were repaired with Klenow fragment of DNA polymerase I and the products restricted with *Eco*RI. These fragments were cloned into the *Eco*RI/*Sma*I sites of M13 mp9 and the endpoints of the deletions determined by dideoxy sequence analysis²⁹. Appropriately sized fragments were removed by *Hind*III digestion, isolated by polyacrylamide gel electrophoresis and ligated into the unique *Hind*III site of pMH-1 (ref. 10). To prepare 3' deletions, the *ss3.6* promoter fragment was cloned into pBR322. The resulting plasmid was linearized with *Hind*III and digested with *Bal*31 exonuclease. The digestion products were restricted with *Bam*HI and the ends were filled-in with Klenow fragment of DNA polymerase. The plasmids were recircularized and HB101 transformants screened for regenerated *Bam*HI sites. One such transformant, determined by DNA sequence analysis to contain a deletion within the promoter extending from -4 to -90 5' to the cap site, was selected for further use. This 883-bp truncated promoter fragment was isolated and *Hind*III linkers were attached, then it was cloned into pMH-1.



Plasmid	Growth condition	% Conversion	Induction	Relative activity
pMH-973	Dark	1.1		
	Light	44.7	40.6	1.00
pMH-722	Dark	1.6		
	Light	13.5	8.7	0.21
pMH-357	Dark	1.9		
	Light	5.5	2.9	0.07
pMH-92	Dark	1.8		
	Light	3.8	2.1	0.05
pMH-973-90	Dark	0.1		
	Light	0.1	0.0	0.00

Fig. 3 The structure of chimaeric genes used to examine the effect of orientation and position of the *rbcS* upstream regulatory sequences when fused to their homologous or a heterologous promoter on photo-regulated *cat* gene expression. □, Chloramphenicol acetyltransferase coding sequences; ■, *rbcS* *ss3.6* promoter sequences; ▨, nopaline synthase promoter sequences; ▽, nopaline synthase polyadenylation signal. **Methods.** The construction of the various chimaeric genes was as follows. In plasmids pSWCAT-R1 and pSWCAT-W1, the 883-bp fragment (see Fig. 1) containing the *rbcS* *ss3.6* upstream regulatory sequences (–973 to –90 bp) was ligated into the *Hind*III site of pMH-92/39 either in the proper (pSWCAT-W1) or reverse (pSWCAT-R1) orientation to the direction of transcription. Plasmid pMH-92/39 was derived from pMH-92 (Fig. 1) by removing the *Hind*III site 3' to the *rbcS* *ss3.6* TATA box. In plasmids pMH-DEF1 and pMH-DEF2, a single copy of the 883-bp fragment to which *Bam*HI linkers were attached was inserted into the *Bam*HI site 3' to the *cat* coding sequence in pMH-92 (Fig. 1) either in the proper orientation to the direction of transcription (pMH-DEF1), or in the reverse orientation (pMH-DEF2). Chimaeric genes containing *ss-nos* promoter fusions were constructed as follows. Plasmid pNCAT-4²¹ was linearized by restriction with *Sac*II and digested with *Bal*31 exonuclease. The digestion products were restricted with *Hind*III, the ends filled-in with Klenow fragment of DNA polymerase, and the plasmids recircularized with T4 DNA ligase. HB101 transformants were screened for plasmids containing regenerated *Hind*III sites and one such transformant, pNCAT-M5, was determined by DNA sequence analysis³² to contain a truncated promoter extending 145 nucleotides 5' to the cap site. This plasmid was restricted with *Hind*II and the 883-bp *rbcS* upstream regulatory sequences inserted. In plasmid pSNCAT-R1, a single copy of the fragment is present in the proper orientation to the direction of transcription; in pSNCAT-W2 a tandem pair of fragments, both in the reverse orientation, has been inserted.



Expression of the truncated *ss-cat* chimaeric genes was determined by assaying levels of CAT enzyme activity in extracts from each independent dark- and light-grown transformed tobacco callus line (Fig. 2). Chimaeric genes under the control of the –973 promoter directed high levels (~40-fold induction) of photoregulated *cat* gene expression in light- versus dark-grown tissue, while deletion of sequences from –973 to –722 caused a dramatic loss of *cat* gene expression, levels of induction being ~20% of the –973 promoter level. Further deletion of sequences from the 5' end of the promoter resulted in additional loss of promoter strength, but did not abolish light inducibility. Truncated promoter fragments containing only 92 bp upstream from the mRNA cap site still directed photoregulated *cat* gene expression, albeit at greatly reduced levels (Fig. 2, pMH-92). The removal of sequences from –4 to –90, which includes the TATA box, resulted in the complete loss of both light and dark *cat* gene expression (Fig. 2, pMH-973-90). This promoter-less upstream fragment was also unable to direct transcription when cloned in the opposite orientation (data not shown).

Since integration of the T-DNA into the plant genome is a random process, variations in copy number and sites of integration might be expected to result in significant differences in levels of expression among individual chimaeric gene constructs. Estimates of copy number ranged from two to five copies per genome and independent transformants expressing the same chimaeric gene construct did not exhibit more than a twofold variation in levels of *cat* expression irrespective of whether the genes were introduced using pGV3851 or pTiC58 (data not shown) as vector. We prefer to accommodate these small differences by presenting levels of induction determined by comparing CAT activity in light- and dark-grown tissue derived from the same transformant.

To determine whether the upstream regulatory sequences required for high levels of photoregulated expression could act independently of orientation with respect to their homologous promoter, the chimaeric genes contained in plasmids pSWCAT-R1 and pSWCAT-W1 were constructed (Fig. 3). These chimaeric genes contain the *rbcS* *ss3.6* upstream regulatory sequences (an 883-bp fragment extending from –90 to –973 bp 5' to the cap site) fused to the truncated *rbcS* *ss3.6* promoter contained on plasmid pMH-92/39 either in the correct orientation to the

direction of transcription (pSWCAT-W1), or its reverse (pSWCAT-R1). In the correct orientation the fusion promoter essentially recreates the 'wild-type' –973 promoter.

The expression of pSWCAT-R1 and pSWCAT-W1 was assayed in light- and dark-grown transformed tobacco calli (Table 1). Chimaeric genes under the control of the truncated promoter fragments contained on plasmids pMH-92 (Fig. 2, pMH-92) and pMH-92/39 (not shown) exhibited only greatly reduced levels of photoregulated *cat* gene expression. However, chimaeric genes under the control of the promoter fusions contained on either pSWCAT-W1 (correct orientation) or pSWCAT-R1 (reverse orientation) showed approximately equal levels of photoregulated *cat* expression to those of the –973 promoter. Thus, these promoter fusions were capable of restoring wild-type levels of transcriptional activity to the –92 promoter. These observations indicate that *rbcS* *ss3.6* upstream regulatory sequences can direct high levels of photoregulated expression independently of their orientation relative to their homologous promoter.

The ability of the *rbcS* *ss3.6* upstream regulatory sequences to function independently of position relative to the TATA box and transcriptional start site was tested by placing these sequences 3' to the coding region of the *cat* gene under the control of the –92 *ss3.6* promoter (Fig. 3). Plasmids pMH-DEF1 and pMH-DEF2 contain chimaeric genes in which a single copy of the *ss3.6* upstream regulatory sequences has been placed at a 3' location either in the proper (pMH-DEF1) or reverse (pMH-DEF2) orientation to the direction of transcription. In dark-grown calli, the chimaeric genes contained on pMH-DEF1 and pMH-DEF2 exhibited levels of CAT activity similar to those observed for a *cat* gene under the control of the –92 promoter alone (Table 1). When these same constructs were assayed in light-grown calli, there was little or no apparent increase in levels of *cat* gene expression above that directed by the –92 promoter. These data indicate that for the constructs tested, the *ss3.6* upstream regulatory sequences cannot confer high levels of photoregulated expression when placed 3' to the TATA box and transcriptional start site.

These observations are similar to those recently reported for the upstream transcriptional regulatory sequences of yeast *GAL* and *CYC1* genes^{24,25}. These yeast regulatory sequences function

independently of orientation 5' to the TATA box and transcriptional start site, but fail to activate transcription when placed 3'. These observations led to the suggestion that the yeast regulatory sequences may differ mechanistically from other known enhancers²⁵. The *rbcS* upstream regulatory sequences may similarly possess fundamental differences. Alternatively, the potential activity of the *rbcS* upstream regulatory sequences may be blocked by elements residing between them and their site of action when in this 3' location^{12,24-26}.

Since sequences within the -92 *rbcS ss3.6* promoter direct low levels of photoregulated expression independently of additional upstream sequences, it may be argued that the upstream regulatory sequences are not involved in mediating light inducibility, but are involved only in determining absolute levels of expression. To test this directly, we fused the *rbcS ss3.6* upstream regulatory sequences to the normally non-photoreponsive¹⁰ promoter from the nopaline synthase (*nos*) gene²⁷ (Fig. 3). In these fusions a *nos-cat* chimaeric gene (Fig. 3, pNCAT-M5) was used containing a truncated *nos* promoter in which all but 145 bp 5' of the mRNA cap site have been deleted. This truncated promoter retains the 'TATA' and 'CAAT' boxes²⁷ and is capable of directing wild-type levels of *cat* expression in light- and dark-grown transformed tobacco calli (Table 1), consistent with previous observations²⁸.

Table 1 Chloramphenicol acetyltransferase activity in extracts from light- and dark-grown transformed calli expressing chimaeric genes used to test for enhancer-like activity of *rbcS* upstream regulatory sequences

Plasmid	Growth condition	% Conversion	Induction	Relative activity
pSWCAT-R1	Dark	1.4		
	Light	53.0	37.9	0.93
pSWCAT-W1	Dark	1.1		
	Light	41.1	37.2	0.92
pNCAT-4	Dark	5.6		
	Light	4.0	0.0	0.00
pNCAT-M5	Dark	3.9		
	Light	4.4	0.0	0.00
pSNCAT-R1	Dark	3.2		
	Light	53.2	16.6	0.41
pSNCAT-W2	Dark	4.6		
	Light	62.7	13.6	0.33
pMH-DEF1	Dark	1.1		
	Light	5.2	4.7	0.12
pMH-DEF2	Dark	1.2		
	Light	4.6	3.9	0.09

Chloramphenicol acetyltransferase activity was assayed and quantitated as described in Fig. 2 legend. Plasmid designations correspond to the chimaeric genes described in Fig. 3. The values presented are derived from experiments using cells transformed with chimaeric genes introduced using the *Agrobacterium* vector, pGV3851.

The chimaeric gene encoded by pSNCAT-R1 contains a single copy of the *ss3.6* upstream regulatory sequences fused to the truncated *nos* promoter in the proper orientation to the direction of transcription and at approximately the same distance upstream from the *nos* transcriptional control sequences as naturally found in the *ss3.6* promoter. In pSNCAT-W2, a tandem pair of the upstream regulatory sequences, both in reverse orientation to the direction of transcription, are present.

In dark-grown calli, the chimaeric genes encoded in pSNCAT-R1 and pSNCAT-W2 each showed similar levels of *cat* expression to those observed for either the wild-type or truncated *nos-cat* gene (Table 1). This suggests that the *ss3.6* upstream regulatory sequences do not alter dark levels of expression of these genes. As expected¹⁰, on transfer to light neither the wild-type nor truncated *nos-cat* gene showed altered levels of *cat* expression. Chimaeric genes containing the *ss-nos* promoter fusions (pSNCAT-R1 and pSNCAT-W2), however, exhibited

dramatic increases in *cat* gene expression in light- versus dark-grown calli (Table 1). Thus, chimaeric genes containing the *ss-nos* promoter fusions exhibit the same photoregulation observed for the *rbcS ss3.6* promoter. Comparison of *ss-nos* promoter fusions and the -973 *ss3.6* promoter construct revealed that the absolute levels of *cat* expression in the light are similar. However, induction levels for both pSNCAT-R1 and pSNCAT-W2 are ~50% of those observed for the -973 *ss3.6* promoter construct, due to the higher levels of CAT activity in dark-grown calli expressing genes containing the *ss-nos* promoter fusions. Placing the *ss3.6* upstream regulatory sequences inverted and in tandem did not affect the levels of induction when compared with a single correctly oriented copy fused to the truncated *nos* promoter. These results demonstrate that the upstream regulatory sequences can mediate photoregulated expression independently of additional transcriptional signals found within the -92 *rbcS ss3.6* promoter.

We have demonstrated clearly that sequences upstream of the *rbcS ss3.6* TATA box both contain information necessary for maximum levels of expression and confer photoregulation. The *rbcS ss3.6* upstream regulatory sequences function independently of orientation when fused to either their homologous or a heterologous promoter. As these upstream regulatory sequences alone are unable to promote expression when joined directly to the *cat* coding sequence (Fig. 2), we presume that the enhanced expression observed from the fusion promoters reflects an increase in the level of transcripts initiated from the normal start site. Experiments to test this directly are in progress. We conclude that the expression of the plant *rbcS ss3.6* gene is regulated by a genetic element that is similar in many respects to the animal viral and cellular enhancers¹²⁻¹⁸. The ability of the *rbcS* enhancer-like element to function when fused to a heterologous promoter will facilitate its characterization. Moreover, the ability of the *rbcS* and related enhancer-like elements to modulate expression via heterologous promoters is likely to prove useful in the genetic manipulation of plant species.

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Reverse transcriptase activity and Ty RNA are associated with virus-like particles in yeast

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The Ty element of yeast represents a class of eukaryotic transposons that show remarkable structural similarity to retroviral proviruses^{1,2}. Recently, these comparisons have been strengthened by a series of observations on the yeast Ty element: (1) Ty transposes via an RNA intermediate³; (2) it contains a sequence (Fig. 1) which, when translated, is homologous to a conserved region found in all reverse transcriptases^{4,5}; (3) a fusion protein encoded by Ty is produced by a frameshift event that is directly analogous to the production of Pr180^{gag-pol} in a retrovirus such as Rous sarcoma virus^{5,6}. Here we identify the reverse transcriptase activity that, until now, has been presumed to mediate Ty transposition and show that it is sequestered in virus-like particles that also contain Ty RNA.

Most Ty elements are about 5.9 kilobases (kb) long with long terminal repeats (LTRs), called delta (δ) sequences, of ~335 base pairs (bp). The major transcript (5.7 kb long) starts at the *Xho*I site in the 'left' or 5' δ and ends ~50 nucleotides beyond the *Xho*I site in the 'right' or 3' δ ^{7,8}. At the 5' δ -internal sequence junction there is a sequence that could base-pair with the 3' end of a tRNA^{Met} molecule⁹, producing a potential primer-template substrate for reverse transcriptase analogous to that used by retroviruses¹⁰. The transcriptional unit is divided into two open reading frames, designated *TYA* and *TYB*, which overlap in different reading phases by 38 nucleotides^{5,6,11}. *TYA* encodes a protein, designated p1, of relative molecular mass (M_r) ~50,000 (50 K)^{6,12}. *TYB* is expressed, via a frameshift event^{5,6}, as a *TYA/TYB* fusion protein (designated p3) of M_r ~190 K¹³. Protein p3(Ty1-15) appears to be processed, and a likely product is a 50 K protein designated p2 (ref. 13). The structure and internal organization of the element used in this study, Ty1-15 (ref. 14), is shown in Fig. 1A.

The striking similarities with respect to structure and expression strategy of Ty and retroviruses suggests that the analogy may include the formation of retrovirus-like particles containing Ty RNA; this is apparently the case for the related *copia*-like elements of *Drosophila* which have been reported to produce virus-like particles (VLPs) containing *copia* RNA and reverse transcriptase activity¹⁵. To test this idea for Ty we used the powerful approach of massive overexpression of a Ty transcriptional unit in a high-efficiency yeast expression vector. (We have used this approach previously to identify Ty-encoded proteins^{11,12,13}.) The entire transcriptional unit of Ty1-15 was manipulated to form a convenient *Bam*HI fragment which was then inserted into the *Bgl*II expression site of plasmid pMA91 (refs 16, 17) (Fig. 1B), the resulting plasmid, pMA91-10, drives Ty expression from the promoter of the yeast phosphoglycerate kinase (*PGK*) gene such that Ty1-15 RNA levels are 20-fold higher than that directed by all the endogenous 35 copies of Ty in the genome¹². The construction and authenticity of pMA91-10 have been described previously¹². If Ty produces VLPs there should be many more present in yeast strain T91-10, containing pMA91-10, than in a control strain, T91, containing vector alone. To test this notion, thin sections of the two strains were examined directly in the electron microscope (Fig. 2A, B). In T91-10 there were large numbers of spherical VLPs of ~60 nm diameter

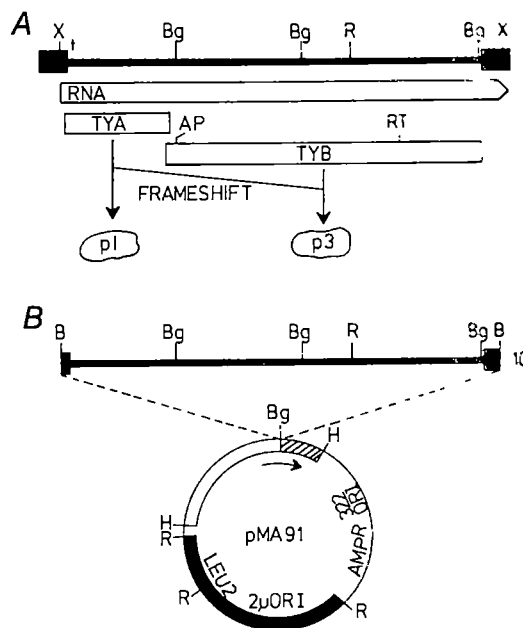


Fig. 1 A, Organization of Ty1-15. Solid boxes, δ sequences. Thick line, internal ϵ region. The major 5.7-kb transcript is indicated. The *TYA* gene extends from nucleotide 299 to 1,619 (ref. 3). The *TYB* gene extends from nucleotide 1,581 to 5,676 (ref. 4 and Fulton *et al.* unpublished data). *TYA* codes for the 50 K protein p1 (Ty1-15)^{6,12,13}. *TYB* is expressed as a *TYA/TYB* fusion protein of M_r ~190 K, p3 (Ty1-15), via a specific frameshift event^{5,6}. AP, region complementary to the 3' end of tRNA^{Met}⁹. AP, region homologous to the active site of acid proteases and retroviral processing proteases³²; RT, region homologous to the conserved region of reverse transcriptases^{4,5}. B, Structure of pMA91-10. The *Xho*I fragment from Ty1-15 was converted to a *Bam*HI fragment (designated fragment 10) as described previously¹² and inserted into the expression vector pMA91 (ref. 16). Plasmid pMA91 is a pBR322 (thin lines) derivative containing a *LEU2*/2 μ origin selection and replication module from pJDB219 (ref. 40) (solid box), the 5' promoter region from the yeast *PGK* gene (open box) and the 3' transcription terminator fragment from the yeast *PGK* gene (hatched box). The *PGK* 5' and 3' regions flank a *Bgl*II expression site. The arrow indicates the direction of transcription promoted by the *PGK* 5' region. X, *Xho*I; Bg, *Bgl*II; R, *Eco*RI; B, *Bam*HI; H, *Hind*III.

whereas in the control strain, T91, there were very few if any. All sections of T91-10 contained particles, which were generally densely packed and represented between ~20% and 80% of the surface area of the sections. The particles appeared to be cytoplasmic and were distributed widely. The difference in numbers of VLPs between the two strains strongly implicates Ty in their formation and we suggest that these particles be designated Ty-VLPs.

If the Ty-VLPs are truly analogous to retroviral particles, they should possess Ty RNA and reverse transcriptase activity¹. Extracts of T91-10 and T91 were therefore fractionated on 15–45% sucrose gradients and the fractions assayed for reverse transcriptase activity and Ty RNA. Initially the reverse transcriptase assay assumed that there would be an endogenous substrate comprising the Ty RNA with a tRNA^{Met} primer annealed to it via the homology at the δ - ϵ junction⁹, although it should be emphasized that there is no direct evidence for this tRNA acting as a primer in this context. A large peak of putative reverse transcriptase activity was found at a gradient position corresponding to a particulate fraction of ~175 S from T91-10 (Fig. 3A); a smaller peak was obtained from the T91 extract. Coincident with this peak of putative reverse transcriptase activity was a peak of full-length Ty RNA (Fig. 3A), suggesting that, as predicted, the 175 S particles contain both the enzyme

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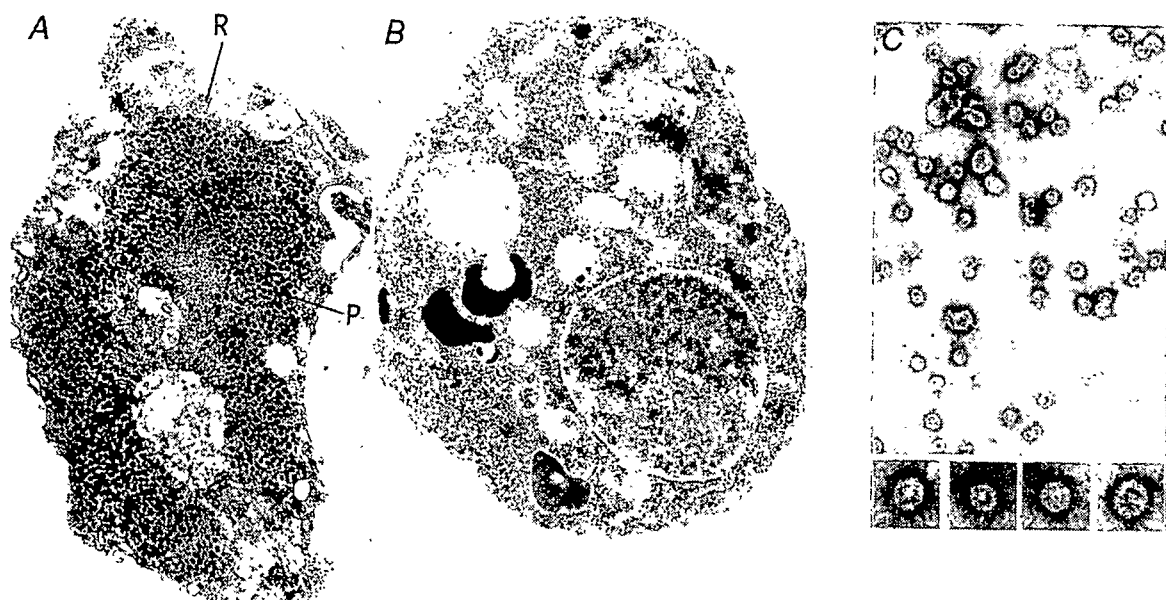
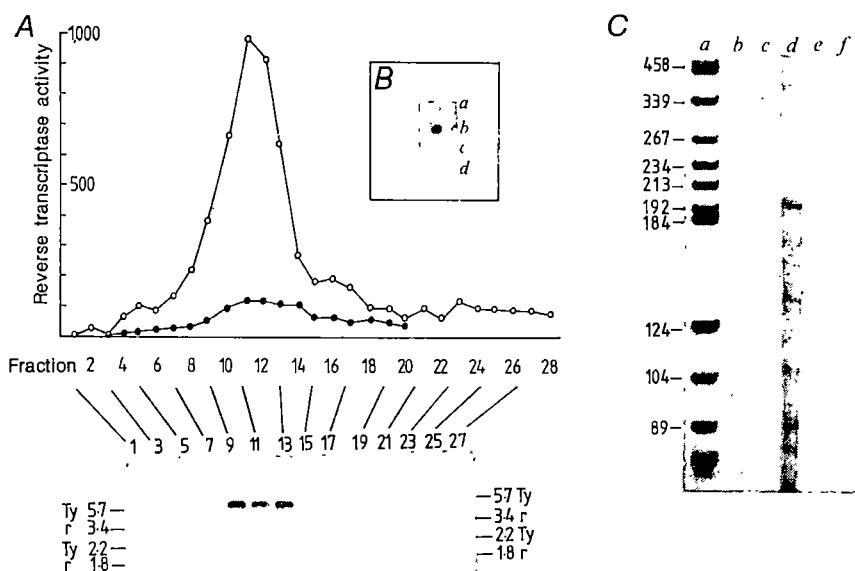


Fig. 2 Electron microscopy of Ty-VLPs. **A**, Thin section of T91-10 ($\times 7,800$). P, Area containing predominantly Ty-VLPs; R, area containing predominantly ribosomes. **B**, Thin section of T91 ($\times 7,800$). Ribosomes can be seen over most of the section. **C**, Uranyl acetate-stained Ty-VLPs from pelleted fractions of a T91-10 extract (low magnification, $\times 30,160$; high magnification, $\times 72,800$).

Methods. Thin sections were prepared according to the method of Byers and Goetsch⁴¹. Purified particles were prepared for electron microscopy by placing 5 μ l of the resuspended pellet on a Formvar/carbon-coated grid and allowing the particles to settle for a few minutes, after which a drop of 2% glutaraldehyde was added, followed by two or three drops of aqueous uranyl acetate. The sections were observed in a Phillips 410 transmission electron microscope.

Fig. 3 **A**, Sucrose gradient fractionation of extracts of T91-10 and T91. The plot shows reverse transcriptase activity in c.p.m. bound to DE81 paper (see below) in fractions from 15–45% sucrose gradients containing material from T91-10 (\circ) and T91 (\bullet). Below the profiles are shown autoradiographs of Northern blots of RNA isolated from the fractions, bound to nitrocellulose and then probed with nick-translated^{1,42} Ty DNA. The positions and sizes of two ribosomal RNA markers (r) and two Ty RNAs (Ty) are marked. **B**, Dot-blot analysis of reaction products. Reverse transcriptase reaction products were hybridized to four DNA spots: a, no DNA; b, Ty DNA (pAT153-11, which comprises pAT153 containing the left-most *Xho*I–*Bgl*III fragment of Ty1–15; see Fig. 1); c, rDNA (pYRG12)⁴³; d, pAT153 DNA⁴⁴. **C**, Labelled endogenous reaction products separated on a sequencing gel: a, M_r markers; b, reaction with low concentration (1 μ M) of cold dNTPs; c, 32 P-labelled dTTP replaced by 32 P-labelled UTP; d, standard reaction (100 μ M cold dNTPs); e, standard reaction followed by treatment with DNase I (50 μ g ml⁻¹; Worthington); f, reaction with no cold dNTPs.



Methods. VLPs were isolated as follows. Yeast cells were grown selectively at 30 °C to a density of $5\text{--}8 \times 10^6$ cells ml⁻¹. The cells were then collected by low-speed centrifugation, washed once in water and resuspended in TEN buffer (10 mM Tris pH 7.2, 2 mM EDTA, 100 mM NaCl) at 1 ml per 1 cells. The cells were disrupted by vortexing with glass beads (40-mesh; BDH) at 4 °C until >70% were broken, then the supernatant was removed and sonicated 10 times on ice for 15 s (6–7 μ m peak-to-peak). The debris was removed by centrifugation (40,000 g for 1 h at 4 °C), and the supernatant layered onto a 15–45% (w/v) sucrose gradient in 10 mM Tris pH 7.2, 10 mM NaCl and spun at 76,300 g for 3 h at 15 °C. Fractions were collected through the bottom of the tube and assayed for reverse transcriptase activity. The endogenous substrate assay was an adaptation of the method of Goff *et al.*⁴⁵. 10 μ l of sample, usually an undiluted sucrose fraction, was added to 20 μ l of an assay cocktail to give the following reaction concentrations (in mM): 50 Tris-HCl pH 8.3, 20 dithiothreitol, 0.6 MnCl₂, 2 MgCl₂, 60 NaCl, 0.1 dATP, 0.1 dCTP, 0.1 dGTP, and 0.06 U ml⁻¹ RNasin (P & S Biochemicals), 0.05% NP40, 0.02 μ Ci μ l⁻¹ [α -³²P] TTP (400 Ci mmol⁻¹; Amersham). This mixture was incubated at 20 °C for 2 h, after which 15 μ l of each assay was transferred to a DE81 filter (Whatman). The filters were washed three times in 5% Na₂HPO₄ for 15 min, once in water, twice in ethanol, then allowed to air-dry. The filters were then counted in a scintillation counter. Products of the endogenous substrate were analysed as follows. ³²P-labelled products were extracted twice with phenol/chloroform (1:1) in TEN, 0.1% SDS and precipitated with 2.5 vol. ethanol, 0.2 M potassium acetate at –70 °C. The precipitate was resuspended in hybridization buffer, added to the DNA filters (see below), incubated overnight at 42 °C and washed twice for 1 h in 2 \times SSC, 20 mM PO₄, 0.1% SDS. Filters were exposed to film at –70 °C with two intensifying screens. RNA isolation and Northern blotting were carried out as described previously¹². DNA was dot-blotted onto nitrocellulose according to Kafatos *et al.*⁴⁶. Detailed analyses (see Figs 2 and 4) were carried out on particles that had been repurified on a second sucrose gradient (25–45%) and then pelleted at 100,000g. S values for the particles in the peak fractions were obtained by re-running the fraction in a Beckman Model E analytical ultracentrifuge.



Fig. 4 Ty-VLP proteins, **A**, Coomassie blue-stained proteins from T91-10 (lane 1), isolated Ty-VLPs (lane 2) and T91 (lane 3). The p1 and p2 proteins in T91-10 and the 70K and 50K proteins from the Ty-VLPs are marked. **B**, DNA-binding profiles of the proteins in **A** electroblotted onto nitrocellulose and probed with 32 P-labelled yeast DNA. Lanes 1-3 same as for **A**. The arrows mark the three major dsDNA-binding proteins present in yeast extracts.

Methods. VLPs were isolated as described in Fig. 3 legend. Pelleted gradient fractions (see Fig. 3 legend) were diluted fivefold with 10 mM Tris pH 7.5, 10 mM NaCl and precipitated for 2 h at -70°C after the addition of 2 vol. cold acetone and NaCl to a final concentration of 0.1 M. The precipitates were resuspended in Laemmli buffer⁴⁷, boiled for 5 min and loaded onto a 9% acrylamide/bis (30:1.6) gel with a 5% stack. The gel was stained with Coomassie blue. DNA-binding assays were performed as described previously¹³ and followed a procedure similar to that described by Bowen *et al.*⁴⁸. Nick-translated⁴² total yeast DNA was used in the assay.

and its template. In addition to the full-length RNA, a 2.2-kb Ty RNA was present in the particles. A Ty RNA of approximately this size has been reported by Taguchi *et al.*¹⁸ but its function is unknown. The peak fractions (10-13 in Fig. 3A) were pooled and examined in the electron microscope (Fig. 2C); Ty-VLPs like those seen in the thin sections were present in the pooled fractions. The particles were relatively pure, the major contaminant being membrane fragments. They appear to be composed of a central core and an outer shell. There were no such particles in fractions 5 and 18.

The putative reverse transcriptase activity was characterized in various ways. The labelled products of the endogenous assay were run on a sequencing gel and shown to be a heterogeneous collection of fragments a few hundred nucleotides long (Fig. 3C). These products, which disappeared totally after treatment with DNase, were substantially reduced if a 100-fold lower concentration of dNTPs was used, and were absent if no dNTPs were added to the reaction. Furthermore, no such products were seen when 32 P-UTP was used as the labelled species. It is likely, therefore, that these products are DNA derived from a sub-optimal reaction. In a dot-blot hybridization experiment which compared the hybridization of the products to Ty, ribosomal and plasmid pAT153 DNA, the products hybridized predominantly to the Ty DNA dot (Fig. 3B). The low degree of hybridization to the ribosomal dot was due to small amounts of contaminating ribosomal RNA throughout the gradient (data not shown). These data show that the activity in the peak fractions incorporates deoxynucleotides preferentially into Ty DNA. Table 1 lists some of the properties of this activity. Incorporation in the endogenous assay is reduced by 76% after pretreatment with RNase, suggesting that a component of the endogenous substrate is RNA; that the activity is only partially sensitive to RNase may be due to protection of the RNA template by the particles. Similar results have been obtained by others^{15,19-21}. An activity profile similar to that of the endogenous assay (Fig. 3A) was also seen with poly(rC)-oligo(dG), poly(rCm)[poly(2'-O-methylcytidylate)]-oligo(dG) and poly(rA)-oligo(dT) primer-template substrates; peak activities for these are shown in Table 1. Poly(rC)-oligo(dG) is a poor substrate for DNA-dependent DNA polymerases, while

poly(rCm)-oligo(dG) is not used at all as a substrate for these enzymes^{22,23}. Furthermore, the endogenous reaction is resistant to treatment with actinomycin D, which inhibits DNA-dependent polymerases. These results suggest strongly that the activity associated with the Ty-VLPs is not a DNA-dependent DNA polymerase. In the poly(rC)-oligo(dG) assay the activity is dependent on both primer and template and does not incorporate significant amounts of 32 P-TTP. These data show that the incorporation of label does not occur via some nucleotide transferase activity. Taken together, these observations strongly suggest that the activity associated with Ty-VLPs is a reverse transcriptase.

Retroviral reverse transcriptase activity is dependent on the presence of divalent cations. Different enzymes show different optima for Mn^{2+} and Mg^{2+} (ref. 24), similarly, the activity associated with Ty-VLPs is totally dependent on the presence of either Mn^{2+} or Mg^{2+} . Retroviral reverse transcriptases are usually liberated from particles by disrupting the viral envelope with detergent²⁰, therefore the activity of the retroviral enzymes *in vitro* is dependent on detergent treatment. When Nonidet P-40 (NP40) was omitted from the Ty endogenous assay there was a reduction of activity of only ~50%. This difference between the Ty-VLP and retroviral systems may be due to the absence of an envelope structure in Ty-VLPs (see below). The Ty-VLP enzyme has a temperature optimum of $\sim 20^{\circ}\text{C}$ although there is not a marked peak of activity; this is compatible with the observation that Ty transposition occurs most frequently at about this temperature²⁵. A detailed comparison of retroviral and Ty enzymes awaits purification of the Ty enzyme and the definition of conditions that reproduce the *in vivo* activity *in vitro*.

As a first step towards the analysis of the protein composition of these Ty-VLPs, we set out to determine whether the particles contained proteins that we had previously recognized as being Ty-specified^{6,12,13}. Peak fractions from the gradients were pooled, repurified on a second sucrose gradient (25-45%) and concentrated by pelleting the particles. The dissociated particle proteins were then run on SDS-polyacrylamide gel electrophoresis (PAGE) alongside a yeast extract known to contain high levels of Ty proteins¹³. Two major protein species of ~70 K and ~50 K are just visible in the stained gel profiles (Fig. 4A). The rather broad 50 K band co-migrates with the previously identified p2 protein in the T91-10 extracts. Protein p2 is one of the few yeast proteins that binds double-stranded (ds) DNA in a blot-binding assay¹³. Therefore, we asked whether the 50 K protein from the purified particles could bind dsDNA. Figure 4B shows that the p2 DNA-binding band that is present

Table 1 Properties of the Ty-VLP reverse transcription

Assay	Variation	c.p.m. incorporated	Activity
Endogenous		2,249	100
	-Mn - Mg	50	2.2
	-NP40	1,086	48.2
	+Actinomycin D	2,259	100.4
	+RNase	547	24.3
	Incubation at 10°C	1,725	76.7
	Incubation at 30°C	1,927	85.6
	Incubation at 37°C	1,443	64.1
	Incubation at 42°C	1,041	46.2
Poly(rC)-oligo(dG)		4,188	100
	- 32 P-GTP + 32 P-TTP	56	1.3
	-poly(rC)	132	3.2
Poly(rCm)-oligo(dG)	-oligo(dG)	172	4.1
		2,505	100
Poly(rA)-oligo(dT)	- 32 P-GTP + 32 P-TTP	41	1.6
	-	2,140	100

The endogenous substrate assay is described in Fig. 3 legend. Additions to the endogenous assay were actinomycin D ($100\text{ }\mu\text{g ml}^{-1}$) and RNase A ($100\text{ }\mu\text{g ml}^{-1}$). Exogenous substrate reactions were the same as the endogenous assay except that either $10\text{ }\mu\text{g}$ of poly(rA) (Calbiochem) and $0.1\text{ }\mu\text{g}$ of oligo(dT) (Collaborative Research) or $10\text{ }\mu\text{g}$ of poly(rC) (Calbiochem) and $0.1\text{ }\mu\text{g}$ of oligo(dG) (Collaborative Research) or $2\text{ }\mu\text{g}$ of poly(rCm)-oligo(dG) (1:1 molar nucleotide ratio) (P.L. Biochemicals) were present in each assay instead of the cold deoxynucleoside triphosphates. 32 P-GTP was used instead of 32 P-TTP in the G-C reactions.

in T91-10 but absent from T91 co-migrates with a band from the purified particles. These data strongly suggest, therefore, that the Ty-VLPs contain p2. The nature of the 70 K protein, however, is unclear.

The discovery of reverse transcriptase associated with a VLP that contains Ty RNA provides the first protein components in a mechanistic model of Ty transposition. Presumably, as is the case for retroviruses, the enzyme catalyses the production of the dsDNA form of the element from the full-length particle-bound RNA template and tRNA^{Met} primer, and then integration could be mediated by an endonuclease. In retroviruses this endonuclease derives from a post-translational cleavage of the primary *pol* product²⁶⁻³¹; it remains to be seen whether the same is true for Ty. Irrespective of the enzymological details, it must be established whether the Ty-VLPs act as units of transposition or are simply the result of an obsolete viral packaging pathway that is perhaps conserved to sequester the potentially chaotic reverse transcriptase away from cellular RNAs.

Recent observations^{3-6,32}, and the data presented here, appear to establish a close relationship between Ty and retroviruses. However, a comparison of the genomic organization of Ty with that of a generalized retrovirus reveals a major difference. Most retroviruses contain three major protein-coding regions: *gag*, which codes for the viral core components; *pol*, which encodes the reverse transcriptase and integrative endonuclease; and *env*, which codes for viral surface components². *TYA* and *TYB* correspond, in position, to *gag* and *pol* respectively, but Ty appears to lack an *env* region. Thus, Ty may be considered a 'defective retrovirus' and its lack of *env* may explain its relatively small particle size (60 nm as opposed to 80-120 nm for retroviruses³³), *env* may have been lost because it was not needed after some ancestral retrovirus found itself trapped within the walled yeast cell. Alternatively, it may not have evolved because selection for such a structure would not have occurred in a particle without an extracellular stage.

Intracellular retrovirus-like particles have been identified in other systems. The most notable are the A-type particles seen in mouse cells³⁴ and the VLPs that contain *copia* RNA and reverse transcriptase activity in *Drosophila*¹⁵. It seems feasible, therefore, that the distribution of this class of genetic elements may be as broad as that of authentic retroviruses. These elements may be responsible for a wide range of genomic rearrangements, gene activation phenomena and the generation of various reverse-transcribed products such as intronless pseudogenes³⁵⁻³⁷ and the human *Alu* repeats³⁸.

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Following submission of this paper, similar results were published by Garfinkel *et al.*³⁹.

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Errata

Electrostatics revindicated classically

R. Nityananda

Nature **316**, 301 (1985).

IN line 16 of this item the word 'contribution' should read 'contradiction'.

Labelling the axes of graphs...

L. Wilson

Nature **316**, 489 (1985).

IN this Scientific Correspondence contribution, the third paragraph should begin: 'The example quoted above requires the observation that if "Radioactivity = 5×10^3 c.p.m." then " $5 = (\text{Radioactivity} \times 10^{-3})/\text{c.p.m.}$ " or " $5 = 10^{-3} (\text{Radioactivity}/\text{c.p.m.})$ " or " $5 = \text{Radioactivity}/(\text{c.p.m.} \times 10^3)$ ". Thus the right-hand sides of any of these last three equations ...'

Corrigenda

Hepatitis B virus contains pre-S gene-encoded domains

A. R. Neurath, S. B. H. Kent, N. Strick, P. Taylor & C. E. Stephens
Nature **315**, 154-156 (1985).

IN Fig. 1 legend there is an error in the one-letter-code sequence of peptide pre-S(12-145) in the text to panels A/B. Position 136 should be a valine residue (V) instead of a tyrosine residue (Y).

A crucial epileptogenic site in the deep prepiriform cortex

S. Piredda & K. Gale

Nature **317**, 623-625 (1985).

THE authors' address was incomplete and should read: Department of Pharmacology, Schools of Medicine and Dentistry, Georgetown University, Washington, DC 20007, USA.

Hypervariable telomeric sequences from the human sex chromosome are pseudoautosomal

H. J. Cooke, W. R. A. Brown & G. A. Rappold

Nature **317**, 687-692 (1985).

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Winning flexibility for research

The largest of the British research councils has produced a corporate plan that dodges all the hard questions. Here is a question that it cannot avoid much longer.

THE variety of names under which British organizations now canvass their hopes and aspirations has become bewildering. In the old days, before 1980, it was by no means unusual that research organizations such as the five British research councils would put out documents called "plans" or, a little more pretentiously, "strategies", from which interested readers might tell what might lie ahead. More recently, it has been the custom for the research councils to prepare each year a document called a "forward look" intended to accompany and sustain their annual requests for government funds. But now, in deference to the British government's conviction that research should be a branch of business, "corporate plans" are all the rage. The Agricultural and Food Research Council was the first in the field nearly a year ago. Now it has fallen to the Science and Engineering Research Council (SERC) to publish (see p.589) a document with such a title, but which is otherwise such a pale imitation of what a commercial corporation would put out under such a heading that there is a danger that the new currency will be debased even before its meaning has been generally understood.

SERC is worried about money, and for a good reason, as it has been for the past several years. The document published this week has its origins in the sense of panic that overtook the council in the autumn of 1984, when it was decided that nothing less than a radical reappraisal of the council's activities would suffice to make ends meet. It had already then been decided that British membership of the European high-energy physics enterprise (CERN) should be reconsidered. SERC off its own bat resolved to decide whether two home-based research observatories were needed to support activities at the overseas observatories, those with real telescopes. And there was to be a reappraisal of the fields of science in which the council would concentrate its future efforts. By all accounts, the steam went out of the enterprise when it was found that the budget for the current year would not be as meagre as at first feared, partly because of a share of the extra £15 million quarried by Sir Keith Joseph, the Secretary of State for Education and Science, from thin air, but at the expense of his political standing with his colleagues. Then, in the summer, came a new chairman and no fewer than seven new members of the council, no more willing to be bound by their predecessors' decisions (and prevarications) than would be a commercial board. The result is a document that still pleads the need for radical reappraisal, which nevertheless still lies somewhere in the future.

Because reappraisal is altogether too important for the health and welfare of the British research community to be left indefinitely on the shelf, and since SERC's problems are not in essence different from those of the other research councils operating in support of basic science in Britain, the new council should face up to the problems it and its predecessors have endlessly defined. The difficulty is that SERC has lost flexibility over the years by the growth of its apparently unavoidable commitments. The commitment to CERN, £35.5 million last financial year, which fluctuates with the value of sterling, is the most conspicuous of these. The two British-based observatories are another constraint on flexibility, and their future may be clearer when the committee on the subject under Sir John Kingman produces its report next year. But the end result is that, out of a total budget of £291 million in 1984-85, SERC was

able to spend only £79 million on research grants and £46 million on research fellowships of various kinds. Where has the rest of the budget gone? The conspicuous commitments are not the only restraints on the council's flexibility. The second largest item in SERC's budget, after research grants, is the cost of the establishment known as the Rutherford Appleton Laboratory (RAL), on the site of the now defunct NIMROD proton accelerator, where some 1,400 professional people provide support services for research in the physical sciences undertaken by the academic community. Should not a truly radical reappraisal consider whether such a large establishment, ostensibly for servicing grant spending which is nearly identical in amount, should continue to exist in its present form?

None of this should be read as a complaint against the laboratory or its staff. By demonstration over the past several years, they have done a splendid job in a variety of important fields. The laboratory is the chief centre in Britain for the development of instruments to be flown in Earth satellites in the pursuit of basic research. It is a computing centre of great value to the British academic community and also a source of innovation in the field. In its own right, the laboratory has an important programme of ionosphere and atmosphere research, a legacy of the merging of the old Appleton laboratory with the old Rutherford laboratory a decade ago. And RAL continues to mastermind some parts of nuclear physics, managing the spallation source that is now the chief source of academic neutrons in Britain. All this, and much else, has been done well. But SERC should ask itself whether such an establishment is, in present circumstances, more of a hindrance than a help.

The arguments for a change at RAL are powerful. As a laboratory whose chief function is to service academic research for the whole of a national research community, RAL is distinguished and probably also unique; nowhere else has such an all-purpose establishment been found necessary or thought wise. The most obvious difficulty is that a commitment on this scale to skilled people, however flexible they may be individually, cramps the council's strategic flexibility. Moreover, it is no longer likely that the work now sited at RAL is all of a kind that must be done centrally because "size and complexity" would overwhelm university sites at which it might be placed. And while SERC's corporate plan boasts modestly of some contract work for industry, when universities are as hungry as they are at present, it is much more likely that technology would be quickly transferred from where it is created if it had sprung from university laboratories, not centralized institutions.

Reading between the lines of the corporate plan, the council plans to be more timorous. There is talk of an agreement with the unions that 10 per cent of the staff at RAL might be on short-term contracts (but the quota has not yet been taken up). What SERC should be planning is a scheme for devolving as much as possible of the work that it does at RAL to universities. Just now, there is a further possibility that some part of RAL might be spun off to the new space centre on which the British government has set its heart. The dangers of such a move are clear in people's minds, but the advantages of the flexibility that could result are greater. The real danger, for SERC, is that it cannot convincingly threaten CERN and its observatories with the knife if the largest of its establishments remains untouched. □

Holding to tenure

The British government and British academics are both misguided on the tenure system.

THE howls of protest that might have been expected after last week's announcement that the British government is to fulfill its threat to abolish academic tenure have been surprisingly muted. Is it perhaps that academics know that it will not be them, or most of them, who will be affected, but their successors? But although the issue is for the time being relatively silent, there is every prospect of trouble when the bill that Sir Keith Joseph promised he would be introducing to the House of Commons later in the parliamentary session begins its passage. For the government's proposals will engender legislation of the worst kind, schemes for putting wrongs (which there are) to rights which are engendered not by a sober consideration of what needs to be done, but by animus. The best outcome would be that one or other of the houses of parliament should throw out what the government proposes, substituting legislation that would give it what it has a right to ask for — and giving academic institutions their just needs at the same time.

Sir Keith Joseph first advertised his plans early last summer. The problem he intends to tackle is the way in which many (but not all) contracts of employment between universities and their academics include the provision that people will have the right to stay at work until their normal retirement age unless previously dismissed for misconduct defined by a narrowly drawn list of misdemeanours. Because the constitution of British universities is defined by means of separate royal charters, legally untouchable by parliament, unless those governed by the charters (which means academics) choose to amend them, parliament and thus the government can act only indirectly, by persuading the Privy Council (the monarch's secretariat) to appoint commissioners with powers to amend the charters one by one. It is a fair guess that this decade will be out before the job is done, which is one reason why an ageing academic workforce may not be too alarmed. But the amendment that the minister now proposes has the further defect that it will apply only to some academics, those newly appointed or those who have the good fortune to be promoted from one grade in the hierarchy to another. So, for a transitional period that will last until the year 2025 or thereabouts, there will be two kinds of British academics — those with tenure and those who do not have it. Tidy government needs something better.

What the government has consistently failed to acknowledge is that academic tenure has a purpose, that of making sure that individual academics are not victimized for unorthodox opinions, a pitfall into which the most imaginative and ultimately the most valuable scholars are prone to fall. Nothing in what the government now proposes will change that state of affairs. What will happen is merely that younger academics in pursuit of unorthodox lines of enquiry will more easily be shown the door by conservative heads of department. Most senior academics, of course, will be too sensible for that, but that is not much of a safeguard against the consequences of the tetchy intellectual enmity that so often blows up in university atmospheres. In this respect, tenure is a necessary and valuable safeguard against intellectual injustice. It is shabby that the British government has let its resentment of the idea that academics "have a job for life" cloud its judgement about the need for a measure of academic freedom. Animus has got the better of it.

But none of this implies that tenure should be inviolable. That is the point at which academics are often wrong. If universities are, or wish to be, self-governing institutions, they must also be free to change course from time to time. If, for example, there should be no students wishing to read Sanskrit at the university at which it is best taught, the university must at least be free to decide whether the department concerned, and those who teach in it, should be dispensed with. Naturally, it would be again be shabby if a university, having seen the demand for Sanskrit fall to nothing, should be able to write out dismissal notices on the

spot, without further consultation. No academic institution seeking respect elsewhere could behave in such a cavalier fashion and then hold its head up (or attract other academics). Even in a free market, scholarship is worth paying for. To ensure that decisions such as these are made in a seemly fashion, two considerations must be satisfied. Universities must be well governed domestically, so that even the victims of hard decisions may feel they have participated in them. And universities must have enough autonomy, financially as well as legally, for good internal government to carry conviction.

How is that to be accomplished? This is where the British government is especially vulnerable to rational complaint. For the past five years, universities have been forced to live financially from hand to mouth. Even at this stage in the current academic year, British universities do not know what subsidy they will be given by the government in the year beginning eight months from now. They will not know for sure until the spring. How, in such circumstances, can universities pretend to themselves, and to those who work for them, that they are self-governing and autonomous? And even if the government is only playing cat-and-mouse with its universities, hoping to keep them hopping with uncertainty, how can it be sure that some of its assets will not give way under the strain? If the government had been more moderate on this contentious issue, it would probably have been able to win what it wants without the fuss now in prospect. What it needs is not a law but a deal, one in which the universities would trade a reasonable self-determined restraint on tenure for a measure of financial security of the kind the government has perversely chosen to deny them. If the government had followed that path, it might even have found falling into its lap the prize it has not yet dared to ask for — the abolition of the doctrine that academics should all be paid the same salaries (at the same age), however successful (or otherwise) the institutions at which they work. □

Poor solstice

Nature's annual weekly break coincides with the break in the calendar, on this occasion by design.

THE New Year festival is of course discriminatory. Its psychological significance is that of the winter solstice; *reculer pour mieux sauter* is the motto, which discriminates against those living in the Southern Hemisphere and even in the tropics. In due course, it will be acknowledged that an equinoctial break (but which?) would be more equitable. Meanwhile, the old northern conventions persist, even to gloomy Nordic reflection on the past and speculation about what lies ahead.

Here is how that train of thought might go. In retrospect, 1985 has been a teasing year. One proof of that is the Geneva summit, which happened; the let-down is that nothing much happened when it did. The US space shuttle has gone up and returned with great regularity (Ariane less reliably), but the seemingly quite recent excitement about journeys within the Solar System has been overshadowed by evidence of the physical weakness of people's hearts and minds.

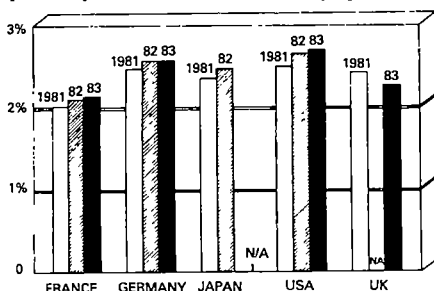
Science in the year past was a fine-textured business. Umpteen more nucleotide sequences cram the databanks, but there are only a couple of extra biogenetic chemicals on the market, and just a handful of gene probes that might spot congenital disease in advance. Most people have learned that, with computers, the software is more important than the hardware — and have taken to complaining about the former. No new fundamental particles have been found (the W and Z belong to 1984), and fundamentalism is out of fashion, in any case.

So will 1986 have a more exciting structure? Predictions being impossible, extrapolations must do instead. The spacecraft Giotto will get Halley's comet (being almost there), the US Congress will be petrified by the prospect of next November's elections, there will be a lot more nucleotide sequences and people will be rightly alarmed by the spread of AIDS, and the consequences thereof. More of that next year. □

UK research councils

Science council thinks ahead

BRITISH academic astronomers are seeking to persuade the Science and Engineering Research Council (SERC) to transfer the staffs of the two UK observatories to university research centres. So much has emerged in the past week, in advance of the publication of the SERC "corporate plan", published earlier this week. The plan says that one of the many questions



Expenditure on R&D as percentage of GDP 1981-83.

still unresolved about its future is that of the two observatories, the Royal Greenwich Observatory at Herstmonceux in Sussex and the Royal Observatory, Edinburgh, whose present functions are chiefly those of providing ground support for British telescopes at La Palma in the Canary Islands and on Hawaii.

Astronomers at the University of Cambridge advocate that SERC should establish a centre for astronomy in Cambridge to which a substantial part of the staff from Greenwich would be transferred. One of the other contenders is the University of Manchester, whose case is based on the commonality of interest between the radioastronomers of Jodrell Bank and those at Edinburgh concerned with the millimetre telescope on Hawaii.

These arguments will presumably be adjudicated by the committee under Sir John Kingman, chairman of SERC until last October, which is looking into the future of the two observatories.

The emergence of these proposals is an illustration of the difficulties faced by SERC in trying to match its future organization to its expected budget. Its plan is more a restatement of familiar problems than a resolution of them.

The starting point is the council's statement that its work is at present limited by the shortage of funds, with the result that many good projects cannot be supported. SERC on this occasion points to increasing competition in basic research not only "by the USA and Japan, but by France and Germany". The plan argues that science has "an exponential dynamic", implying that discovery creates new needs, and says that external constraints on its operations at present are "too severe".

For the immediate future, SERC says

that it hopes by 1989 to release an extra £18 million a year from its own resources. The most urgent use of the funds, the plan says, is for the direct support of universities. As well as seeking a reduced subscription to the CERN laboratory at Geneva, SERC intends to reduce spending on support for high-energy physics by a fifth. On space research, the council says that it will concentrate its support for the European Space Agency on projects in basic research, and that its intended participation in the British National Space Centre will not persuade it, "at least for

the time being", to increase spending

Among the other casualties of planning within restraint are the proposed upgrading of the tandem accelerator at Oxford. Ultimately, SERC says, it will also want to reduce its involvement in the Institute Laue - Langevin at Grenoble, although it hopes before then to have reached agreement with other European research councils for international participation in the Spallation Neutron Source at the Rutherford Appleton Laboratory in exchange for British participation in the European Synchrotron Radiation Facility. □

British research

A little more all round

SIR Keith Joseph, British Secretary of State for Education and Science, this week announced the science budget for the next financial year (see table). Sir Keith has accepted the advice of the Advisory Board for the Research Councils (ABRC), which each year tells the government how it thinks the money

Government expenditure on British research (£ million)

	1985-86	1986-87	Increase (per cent)
AFRC	50.3	52.7	4.7
ESRC	23.6	23.6	0
MRC	122.3	128.3	4.9
NERC	67.3	70.3	4.5
SERC	298.0	315.5	5.9
British Museum (Natural History)	16.2	17.2	6.2
Royal Society	5.9	6.4	8.5
Others		0.6	
Total for 1986-87		614.6	

should be divided. The only part of ABRC's advice that Sir Keith rejected was that the Department of Health and Social Security should bear the full costs of establishing a centre for coordinating epidemiological research on acquired immune deficiency syndrome (AIDS). Although the health department may pay up to £0.3 million to the centre, Sir Keith has firmly told the Medical Research Council (MRC) to meet the balance of the costs of the centre from its allocation.

The total science budget for 1986-87 includes an extra £15 million that Sir Keith pulled out of his hat last month. ABRC has recommended, and Sir Keith agreed, that of the new money, the Agricultural and Food Research Council (AFRC) should receive £2.5 million for "new initiatives in its institutes", MRC £2.5 million for research grants and programmes, the Natural Environment Research Council

(NERC) £1.9 million, the Science and Engineering Research Council (SERC) £6 million to support "strategic research of industrial relevance" and the rest will enhance ABRC's "flexibility margin".

ABRC intends to produce a strategy document next year to take account of the corporate plans of each research council (see above). It will also change its normal schedule so that it will consider both the overall financial position of the science budget and the relative positions and claims of the individual research bodies in one exercise in March 1986.

The only body not to receive an increase in funds for 1986-87 in Sir Keith's statement is the Economic and Social Research Council (ESRC). The council is unlikely to be too distressed at the news; this is the first time in four years that its budget has not been cut. ESRC has taken seriously its brief from ABRC to toughen up; last month it took the controversial decision to blacklist any institution in which ten per cent of research students failed to submit a thesis within four years, and deny them the right to apply for any more grants for two years. Fourteen such institutions were blacklisted and given until last week to appeal. ESRC has now considered those appeals, and this week announced that five institutions have been reinstated for various reasons, including inaccurate statistics, failure to inform ESRC of withdrawal and two administrative errors.

Kings College London (KOC), Leeds Polytechnic, the University of London Institute of Education, University College of Wales (Swansea) and UMIST have gained reprieves. But Queen's University Belfast, the Universities of East Anglia, Liverpool, Aston and Dundee, Manchester Business School, Sheffield Polytechnic and Paisley College of Technology have failed to persuade ESRC to reverse its decision. Maxine Clarke

Strategic Defense Initiative

Congress questions UK pact

Washington

DESPITE high hopes in Europe that President Reagan's Strategic Defense Initiative (SDI) might lead to thousands of millions of dollars of SDI contracts for European scientists and engineers, the Europeans are unlikely to see much more than about \$300 million in total, according to congressional testimony last week by John Pike of the Federation of American Scientists, an unofficial pressure group. Pike's analysis, based on an examination of existing SDI contracts, identifies six major barriers to European cooperation and warns that the political repercussions due to failed expectations will reduce the already hesitant and patchy support for SDI in Europe.

SDI is expected to let contracts worth in total about \$30,000 million in the five years to 1990. But almost half of this will be off-limits to European countries because of articles and "agreed statements" in the Anti-Ballistic Missile (ABM) treaty of 1972, which prohibit transfer to other states of not only ABM systems and their components but also technical descriptions and blueprints specific to such systems. The US administration has repeatedly said that it is conducting SDI research in full compliance with the ABM treaty, and British support for SDI is conditional on continuing compliance.

Pike believes the treaty will prevent European participation in major SDI projects such as sensors, rocket interceptors and directed-energy weapons. Instead, participation would be restricted to basic research and development only of very small devices and subsystems, for which the dollar value would be "trivial".

Pike's second major impediment to cooperation is the limited capabilities of European companies, which in his view puts a further one-third of SDI beyond their reach. Pike pointed out that, in contrast to the very limited experience of European companies, the United States has spent \$50,000 million on ABM research over the past 30 years.

A twelve-year-old US defence procurement regulation prohibits contracting with foreign sources if a US source is equally competent and willing to do the work; exceptions appear to be allowed only if the foreign government concerned reimburses the US government for the work. The record so far is not encouraging: none of the 1,000 SDI contracts so far let have been to non-US companies (there is one small subcontract in Britain) and there were similarly no contracts for non-US companies among the \$3,000 million spent by the US Army on ballistic missile defence between 1975 and 1985.

Having disqualified non-US companies from \$24,000 million of SDI work on treaty and technical grounds, Pike went on to tell a subcommittee of the House of Re-

presentatives' Committee on Banking, Finance and Urban affairs that a further \$5,000 million was excluded because its commercial potential would mean keeping it in the United States or because of inherent geographical restrictions. Of the \$1,000 million left over that would be available for foreign competition, Pike estimated that a maximum of 30 per cent is likely actually to go overseas.

The hearing was held shortly after the announcement of the signing of a memorandum of understanding between Britain and the United States on SDI. As the terms have not been made public, the likely effect of the agreement is hard to gauge. But Representative John LaFalce (Democrat, New York) told Dr Gerold Yonas, chief scientist of SDI Organization, that he believed the purpose of the US administration in obtaining the agree-

ment with the British was political in that it was expected to foster European political goodwill. LaFalce warned that the United States might "build itself up for a fall" if the agreement created false hopes.

Concern about the effect of the agreement on US employment and the economy was evident in aggressive questioning of Yonas by Representative Bruce Vento (Democrat, Minnesota). Vento repeatedly asked how there could be open competition for research contracts if even a non-specific commitment had been made to support research in Britain. LaFalce questioned whether the United States should be entering agreements that might mean US taxpayers' money generating commercial spin-offs to benefit Europe. Questions were also asked about the confidence the administration had that classified material would be kept secret in Britain. Yonas replied that one of the purposes of the intergovernmental agreement was to control classified information centrally.

Tim Beardsley

European military technology

France veers towards integration

THE US Strategic Defense Initiative (SDI) is an imperfect shield in response to which the Soviet Union need only "sharpen its foil", French scientists claimed this month at the second open "science and defence" meeting organized by the French government.



But the canny French do not ignore the implications of the US programme. Did they not launch the Eureka programme of European collaboration in high-technology products in response to SDI? Last week, M. Roland Dumas, the French foreign minister, said that Europe should now also pay attention to the development of a military counterpart to Eureka. Dumas thus mildly echoed the somewhat stronger proposal a week before of the previous President of France, Valéry Giscard d'Estaing, that Europe needs not only a military Eureka, but its very own "star wars" shield.

Dumas did not go so far. Nor did the other participants in the meeting, held at the Ministry of Defence's own grande école, the École Polytechnique. The technical aspects of SDI were thoroughly rehearsed — a defensive satellite system

would need twenty times as many satellites as could be used to repel an attack, 95 per cent of the system being likely to be out of range, while the Soviet Union could launch missiles in convoys to saturate this usable 5 per cent. The hardening of launchers, a reduction in the boost period, rotation of the vehicles and other measures could increase the power requirements of directed-energy weapons twentyfold, people argued. And cruise missiles deep in the atmosphere could avoid the system altogether.

Dumas said that SDI has nevertheless already achieved one thing — the "scattering" of Europeans, with each nation reacting differently to the challenge both technologically and politically, despite the counterbalancing effects of Eureka. There must in particular be an effort at cooperation in military technology within Europe, Dumas said, overlooking the recent commercial and diplomatic conflict between France and Britain over the supply of advanced battlefield communications technology to the US Army. Dumas went on to say that it is time "to provoke a political effort among governments . . . very soon in the coordination of military and industrial policy". Dumas was arguing, indeed, for a truly European "military/industrial complex" of the kind that has stimulated much basic and applied research in the United States and which, so far, is lacking in Europe. Strange again, perhaps, for the foreign minister of a Socialist government which in rosier days was arguing for the banning of nuclear tests and a curbing of the world arms trade, of which France is now the leader behind only the United States and the Soviet Union.

Robert Walgate

Japanese ethics

Edging towards transplants

Tokyo

HEART transplants are likely to begin in the near future in Japan following the preparation of a new definition of "brain death" by an advisory panel of the Ministry of Health and Welfare. Only one heart transplant has ever been carried out in Japan: that was 17 years ago and caused a major outcry, including demands that the doctor responsible be tried for murder. Similar demands for prosecution were again made this year when surgeons performed a combined liver and pancreas transplant operation at Tsukuba University.

One major factor that facilitates opposition to transplants is the lack of an officially recognized or widely accepted definition of "brain death". A patient who is brain dead has lost all brain activity and has no possibility of recovering. But the use of "artificial lungs" and drugs to maintain blood pressure can keep the patient breathing and his heart beating, often for periods of many weeks. Transplants of "living" hearts and lungs from these patients can save other people's lives.

Without continuous artificial respiration, a brain-dead patient's heart will stop very quickly; a patient whose brain stem still functions and who can breathe without artificial aid is a different matter. Whether or not the life of these latter patients can be legally terminated has recently been the focus of attention in the West. Brain death has, however, already been accepted as a legal criterion of death and has made transplants of heart and liver almost commonplace. Japan on the other hand is still a long way from accepting brain death as death of the person.

Until now, a set of criteria of brain death recommended by the Japanese Society of Electroencephalography has been in general use but each hospital has modified it as it sees it. There has been general reluctance to remove organs from brain-dead patients in order to conduct transplant operations; instead, patients have been maintained for weeks or months until the heart stops beating and the patient is recognized as legally dead. This has both inhibited the performance of transplant operations and tied up expensive medical facilities.

The new definition is considerably more straightforward than the earlier one. It was worked out by a panel of eight neurologists who examined the medical records of more than 700 cases of brain death. Five symptoms are now taken to define brain death: deep coma; loss of spontaneous breathing; enlargement of both pupils to more than 4 mm; the loss of reflexes governed by the brain stem; and flat brain waves, all lasting for at least six hours. Certain cases are excluded — those involving young children whose powers of recovery may be exceptional and those

caused by poisons.

Armed with the new definition, doctors will have a much clearer guide to the propriety of recognizing a patient as dead and the number of transplant operations is likely to increase. But the new definition is only a beginning and by no means clears away all obstacles to transplant operations. The ministry has no intention of taking the next step and preparing a new law that would recognize brain death as a legal definition of death. Doctors responsible for brain-dead patients will thus still be in an ambiguous position and acceptance by family members will be required before brain death can be agreed to constitute the death of an individual. The extent to which brain death is truly recognized and the facility with which transplants will be carried out will thus depend on how quickly brain death is accepted as "death" by ordinary citizens. At present, surveys show that less than a third of the population are ready to take this step.

One obstacle is a suspicion that doctors

are over-eager to perform transplants. Partly to allay such fears, two doctors on the advisory panel involved in transplant research resigned before its conclusions were released. It was thought that those known to favour transplants would not influence the panel's conclusions.

A separate difficulty lies in public attitudes towards organ donation. Despite figures that show that Japan has fewer believers in religion than any other non-communist country (if belief in some form of after-life or reincarnation can be taken as a definition of religion), there remains in Japan a peculiar resistance to donating organs. Even kidneys are in short supply, although transplants may be made from patients whose hearts have stopped beating. Quite why this is so, nobody is sure; commentators point to everything from the influences of Buddhism and Confucianism to a lack of public information campaigns (see *Nature* 313, 338; 1985).

Several competing hospital groups are known to be eager to boost their reputations by performing the nation's "second" heart transplant, and the new report will certainly improve their chances of doing so without censure. **Alun Anderson**

Space telescope

Discretion dispute on viewing time

Washington

US ASTRONOMERS are divided over a proposal to give scientists at the Space Telescope Science Institute (STSI) preferential treatment in the competition for viewing time on the Hubble Space Telescope. The proposal would ensure that principal investigators at STSI got at least 5 per cent of viewing time by using some of the "director's discretionary time" set aside for maintenance, calibration and unforeseen "targets of opportunity".

The proposal has been formally presented by STSI staff to several space telescope advisory committees. The preferred option is that up to half of the director's discretionary time (which amounts in all to 10 per cent of observing time) would be used by STSI principal investigators who failed to win time in the open competition for observing time. Only as much discretionary time as necessary to bring STSI principal investigators up to 10 per cent of the total observing time would be allocated in this way.

Concern about the proposal has been expressed in a letter sent to Dr Lyman Spitzer Jr, chairman of the Space Telescope Institute Committee, and signed by Dr Jeffrey Linsky, chairman of the Astronomy and Relativity Management and Operations Working Group. The working group says the STSI proposal is contrary to the spirit of the initial agreement that led to the establishment of STSI. The letter goes on to acknowledge that the proposal would solve a morale problem at STSI but questions whether the suggested

figures adequately reflect the proportions of competent investigators at STSI and elsewhere. At many other national astronomy facilities, in-house scientists compete in the usual way and succeed in getting an adequate proportion of viewing time, from 15 to 40 per cent of what they ask for.

Linsky's committee also disputes that the proposal would not delay observations by other guest observers. It seems likely that some of the special capabilities of the telescope, for example the ability to track moving objects such as a detail on a planetary moon, will not be completely calibrated at launch, and all of the director's discretionary time may be needed for that purpose.

The reason cited by STSI to support preferential access is that it would be in the general interest of the project to ensure that some scientists at STSI are also observers. STSI argues that its scientists are at a disadvantage in the competition for viewing time because they are working flat out to complete STSI's data handling and control systems in time for a scheduled August 1986 launch and so have been unable to devote enough time to preparing observing proposals.

Dr Riccardo Giacconi, director of STSI, said that while STSI scientists nominally spend 50 per cent of their time on research, in practice many spend only 20 per cent. Giacconi said a system similar to that being proposed is used at Kitt Peak National Observatory. **Tim Beardsley**

Electron-beam lithography

How to make chips to order

Menlo Park, California

THE Stanford Research Institute (SRI) International, the commercial contract research institute, claims to have carried the technology of electron beam lithography to the point at which the technique can be used for making individual semiconductor microchips, not simply the masks used in semiconductor mass production. Those responsible say that the technique has hitherto been too slow to be used in mass production, where optical lithography remains the chief technique. But SRI will need a commercial partner — and millions of dollars — to commercialize its machine.

According to Dr Ivor Brodie, director of SRI's physical electronics laboratory, the growing demand for bespoke microchips, both in silicon and gallium arsenide, has helped to make electron beam lithography a commercial proposition. SRI is now looking forward to a market that could be worth \$1,000 million a year by 1992. Brodie says that the demand for bespoke chips was not foreseen. "People want a particular integrated circuit for a particular purpose, and do not want others to copy it", he says. Instead of relying on general-purpose devices programmed for particular functions, he says, "people are now doing the programs, as it were, by hard-wiring".

The SRI machine is certainly quick. A semiconductor wafer 200 mm in diameter, and typically carrying 1,200 separate chips, can be "written in about a minute", according to Brodie, who says that with existing electron beam machines, it takes between half an hour and an hour to "write" a mask of the same size.

The electron beams in the SRI design are fired from an electron gun, focused through a screen of lenses at zero volts to the wafer being processed, which is itself maintained at a higher voltage. The multiple beams can be switched off and on by adjusting the voltage. By the use of several beams, it is possible to etch an identical pattern on several different parts of a semiconductor wafer, thus allowing several identical integrated circuits to be created simultaneously.

NASA Lewis Research Center and the Naval Research Laboratories have led SRI to develop custom-made microchips that could miniaturize CRTs. Capp Spindt, senior research engineer leading the project, says that NASA's interest was originally inspired by the need for a source of high current density for its satellite communications. Thermionic emission on cathodes were inefficient, needing heat to excite electrons from an element into the vacuum to create the electron beam. SRI's approach is based on field emission techniques in which electrons are produced by tunnelling to the surface of the metal on which is induced a high

electric field. The process is efficient.

Using this foundation, SRI has developed microchips which produce very-high-current densities — more than 60 A cm⁻² to 100 A cm⁻² or about three times the current densities normally expected of thermionic emission. The design, says Spindt, is based on the well-tried methods adopted for years in the semiconductor industry — a sandwich of metal, silicon oxide and silicon (MOS).

Using electron beam lithography, the researchers were able to etch out the design creating a pattern of holes in the substrate into which they could insert a metal, the source of electron emission. "You have this metal film with a hole in it. You

have an insulator spacing it . . . and the ground plane silicon you can work from. And it's very closely spaced. Then we developed a technique for growing a very sharp point which rests on the silicon."

The distances between these holes are about 2 µm, giving densities of more than 1.2×10^6 tips per cm², and arrays with more than 10,000 tips have been fabricated. Very high current densities result when the device is electrically excited. The next stage in the development is to explore how the technique can be used to enhance the designs of displays. The high current densities are expected to make any CRT picture brighter — and therefore ideal where the environmental light is at a high level, as in a cockpit/flight-deck — and because of the compactness of the silicon, to reduce substantially the width of any display.

Bill Johnstone

Creationism

California sells out on textbooks

Washington

EVOLUTIONISTS in California were bitter last week about what they call a "sham" hearing held by the State Board of Education that approved revised biology textbooks for state schools despite "hundreds" of factual errors and weak coverage of evolution. Revisions made by the publishers to take account of criticisms by the state's curriculum commission introduced more errors than they eliminated, according to William Bennetta, who has been working for better treatment of evolution in textbooks.

The revisions were made after the curriculum commission recommended in September that the books, for twelve- and thirteen-year-olds, should not be accepted because of their avoidance of evolution and inadequate treatment of human reproduction and ethical questions. Publishers are anxious not to offend the strong US creationist lobby, which has threatened to sue in California over the

books now approved. California represents more than 10 per cent of the US textbook market.

A spokesman for the state board said that the books had been greatly improved by the revisions. Publishers had threatened that a failure to adopt the revised books last week would have made it impossible for books to be produced in time for the start of the academic year next September; because textbooks are adopted in California on a six-year cycle, a delay could have held up planned improvements to books in other subjects. Dr Bill Honig, state superintendent for public instruction, said he believed the books now to be the best in the country. A final review by a curriculum commission subcommittee would be able to eliminate any factual errors in the books that were identified at the hearing.

Bennetta and his supporters are not impressed with this promise, pointing out that the subcommittee has no biologist among its members. Most of the books were not altered extensively, as the evolutionists thought necessary, and even those that made significant changes avoided open acknowledgement that evolution is the received wisdom, often using such terms as "many scientists feel" as qualifiers.

The evolutionists complain that the revised books were deliberately made difficult to examine (one copy only was exhibited, for 12 working days, in the state capital) in order to minimize the risk that errors and weaknesses would be exposed. Bennetta's favourite example is in *Health Life Science*, published by Heath and Company, where a statement in the original version that "many scientists believe that dinosaurs were the ancestors of reptiles" has been replaced by the assertion that scientists classify dinosaurs as the ancestors of reptiles.

Tim Beardsley

Aleksandrov's fate?

ALMOST nine months after the disappearance of Soviet computer scientist Vladimir Aleksandrov in Spain, the Soviet Academy of Sciences last week said that he had disappeared without trace in Madrid on 1 April on his way home from an international conference of mayors of "nuclear-free" towns. Diplomatic representations had received "no clear reply". The academy did not explain why it had been officially silent for so long. But Soviet unwillingness to discuss the disappearance has, undoubtedly, fuelled rumours circulating in the West that Aleksandrov, co-author of the Soviet nuclear winter computer model, had been intending to defect, but was prevented by Soviet security men.

Vera Rich

French space

Will US Congress fill vacuum?

THE French space agency, CNES, plans to spend more on basic science, lifting the proportion of its funds from 6–7 per cent now to 10 per cent by 1990. But this is only a target, according to research director Isaac Revah last week. One of the imponderables is whether US participation in the joint Topex/Poseidon oceanographic satellite, France's biggest project so far in basic space science, will survive the automatic budget-cutting mechanisms to which the US government is now committed (see *Nature* 318, 495; 1985).

So CNES is hatching contingency plans for Poseidon, the French half of the project. British or Scandinavian participation is being mentioned as an alternative. But the possibility of collaboration with the Soviet Union, for which there are many

CNES precedents, is "certainly not" on. The Poseidon satellite instruments, consisting of a high-resolution altimeter, a troposphere radiometer and positioning equipment capable of fixing orbital positions to within 10–15 cm, have an obvious military interest.

The US half of the project, Topex, has also thrown up problems of secrecy. The purpose of these instruments is to infer ocean currents from measurements of ocean topography. Nominally, the project is a civil enterprise. But the US Department of Defense has slapped restrictions on the details of the instrumentation. France, through CNES, has retaliated by offering the United States only black-box descriptions of what the Poseidon instruments will do. If the US National Aero-

navics and Space Administration (NASA) fails to get its money, Poseidon will be flown on a French Earth observation satellite, one of the SPOT series, in 1990–91. "We don't need technology transfer in this field", Revah says.

The French space programme for the next few decades, now becoming clear in its outlines, is diverse and ambitious. In basic science, according to Revah, more attention will be paid to Earth science, but not at the expense of astronomy, supported mainly through agreements with the Soviet Union. On the hardware side, France is committed to the development of the Hermes space shuttle, the cryogenic third stage of Ariane, and participation in the European contribution to the US space station, the separate vehicle called Columbus.

For the rest, the following projects and programmes are on the cards.

- A satellite called Magnolia for precise measurement of the Earth's magnetic field, possibly to be built before 1995. (A similar project for the measurement of gravitational fields, called Gradio, has encountered technical snags and may be put back.) Beyond that, CNES prefers to talk of programmes rather than particular satellites, but there is an inclination to concentrate on measurements that will help to define the hydrological cycle in the troposphere, with obvious applications in climatology. By 1995, the officials say, it may also be possible to make direct measurements of the ground motions expected from plate tectonics and at geological faults, either by the comparison of successive high-resolution photographs or with the help of fixed ground beacons.

- In astronomy, priority will probably be given to Franco-Soviet collaboration on Vesta, a mission to Venus and the asteroids, that will cost France FF600 million. But there is a host of other projects, suggested by the French community, competing for CNES funds. These include a low orbiter for the study of the internal structure of Jupiter, a Mars rover that would look for traces of water, a radio-interferometer called Trio and participation in the proposed European Space Agency (ESA) high-resolution spectrographic X-ray telescope. The only certainty is that CNES will not be able to support all these projects.

- On manned spaceflight, CNES hopes to arrange a long-duration space flight with the Soviet Union so as to engage the French space medicine community; as always, it is looking for industrial interest in the exploitation of microgravity environments.

This year, French spending on these activities will have amounted to FF150 million in bilateral programmes, almost entirely on instrumentation, plus FF200 million on the ESA science budget. This makes a total of FF350 million, compared with the goal for 1990 of FF600 million.

Robert Walgate

Soviet science

Making policy by TV camera

A REMARKABLE innovation in television programming, allowing public questioning of Soviet government officials, seems to have been part of the preparation for the Soviet Union's 27th Party Congress in February. On 29 November, Soviet television stations broadcast a phone-in with viewers' questions about "accelerating scientific and technical progress", a keynote phrase of all Soviet economic documents of the past decade.

The panel included Academician Anatolii P. Aleksandrov, president of the Soviet Academy of Sciences, two vice-presidents of the academy (Evgenii P. Velikhov and Yuri A. Ovchinnikov), two deputy prime ministers (Ivan S. Silaev and Guri I. Marchuk) and the chairman of the State Committee for Discoveries and Inventions, Ivan S. Nayashkov. The producers seem to have created an atmosphere of spontaneity, but since it had also been possible to telephone questions in advance, and since the questioners' voices were never heard, it is impossible to know how many replies were genuinely unprepared.

Nevertheless, the programme does seem to have reflected genuine public criticism and doubts. Here are summaries of some of the exchanges:

Q: Why are the power sets of the Leningrad, Ignalina and Chornobyl nuclear power stations built in five different — and flawed — designs?

A: We're working on it.

Q: Why does it take more than 12 years for a "practical invention" to be introduced into the economy?

A: It takes only six years, and we are trying to reduce this time.

Q: What are personal computers, and won't they make people "unlearn" how to count? (This question was from a school-

boy, presumably too young to have started the new compulsory computer studies course.)

A: On the contrary, they expand the human capacity for thought.

Q: What is being done to overcome "high-handed administration" and "the cult of the director" in certain scientific establishments?

A: The powers of directors are now being widened, but we have the means to counteract the negative features of high-handed administration.

Some questions, however, seem to have been specially picked to allow the panel to expatiate on new plans and proposals — the concentration in the new Five-Year Plan on machine tools, the establishment of biotechnology centres in rural areas or of a laboratory to monitor how the young generation in schools accept computer technology, and "whether it will do any harm to their development".

A question to Academician Ovchinnikov in his role as chairman of the All-Union Society for the Struggle for Sobriety itself answered one question that has been puzzling many Soviet citizens, not to mention Soviet-watchers abroad — why have scientists in particular been coopted in such force into the anti-alcohol movement?

Ovchinnikov's answer is that the acceleration of scientific and technical progress requires increased labour discipline, that is, the elimination of drunkenness. Ovchinnikov, himself, it appears, is in favour of total abstinence.

Other questions related to recent policy decisions such as the merging of the medical and microbiological industries, and the commitment to thermonuclear power emphasized at the Geneva summit.

Vera Rich

British research

Catalogue of steady decline

ALTHOUGH declining British government support for civil research is offset by an increase in support for military research, this has not prevented a decline both of the proportion of the Gross Domestic Product (GDP) spent on research and of Britain's standing relative to other industrial nations in civil research spending. This is the simplest lesson to be drawn from the *Annual review of government funded R & D 1985*, published earlier this week (HMSO, £9.50).

The survey is the fourth in the series produced since the House of Lords Select Committee on Science and Technology recommended in 1981 that there should be such a document. It is also the last to be produced by Sir Robin Nicholson, the scientist member of the Cabinet Office who will be leaving in the new year to become research director of Pilkingtons, the glass manufacturers.

Nicholson obviously hopes that he established a tradition that will survive not only his office but general elections yet to come, explaining how future editions will improve on the quality of the data (which is at present variable, especially on topics such as manpower). One guarantor of survival is that the survey is entirely free of value judgements, and that government departments' own estimates of their research spending under various categories are normally entered as supplied.

The survey is largely based on figures for spending by government departments covering the last financial year (to last April) together with the three immediately previous years and plans for the present and the two succeeding years (as announced last February). The figures, such as they are, for industrial spending on research and development come from a survey carried out by the Department of Trade and Industry.

In the current year, according to the survey, government spending on research and development is at a shallow peak, amounting in real terms (and 1983-84 prices) to £4,137 million. On present plans, the total is expected to decline from now on, to £3,966 million in 1987-88. Within this total, however, defence spending on research and development, now just over a half at £2,077 million, is expected to increase modestly to £2,181 million in 1987-88 (again at 1983-84 prices). But even these figures make the conventional but increasingly doubtful assumption that about 30 per cent of spending on higher education may be counted as research expenditure.

Nicholson emphasized earlier this week that the international comparisons suggested by the survey are made uncertain by the extent to which the data cannot easily be compared. But according to data collected by the Organisation for Econo-

mic Cooperation and Development (OECD), Britain stands out as the only major member country in which total spending on research and development (by industry as well as government) has actually declined in recent years (by 0.9 per cent in 1983, the latest year for which a comparison is attempted). □

US panel split on guidelines

Washington

AGREEMENT was finally reached on 4 December on policy guidelines for the safe introduction of recombinant DNA technology by a working group of the Organisation for Economic Cooperation and Development (OECD). The guidelines have now only to be formally approved by OECD's Committee on Science and Technology Policy before being published.

The drawing up of the guidelines, which has taken two years, spawned dissent between US agencies which led to the cancellation of a meeting of the working group in August and expressions of concern by US senators. At the meeting earlier this month at OECD's headquarters in Paris, the US delegation offered a radically changed version which omitted a section of a previous draft dealing with large-scale industrial practices. The compromise document now agreed represents "a merger of the best of both versions", according to Dr Frank Young, commissioner of the US Food and Drug Administration (FDA), who secured the eventual agreement between US agencies. Dr Henry Miller of FDA, whose involvement in the project generated controversy, said he was "absolutely delighted" with the results.

The confusion over the US position delayed completion of the guidelines by 4-6 months. The representative of the Environmental Protection Agency who earlier coordinated US work on the project boycotted the latest meeting.

The document now agreed endorses the use of agreed standards of good industrial large-scale practice for genetically engineered organisms deemed to represent a low risk; many OECD countries had been waiting for this recommendation to cite as justification for their domestic policies. Higher-risk organisms can be safely handled with established containment techniques. When considering environmental releases of genetically altered organisms, the document says risks can be evaluated in the same way as for other organisms, and recommends a step-by-step approach of progressively expanding the containment through small-scale and then large-scale field tests before allowing full release.

Tim Beardsley

Deep-sea drilling

Britain rejoins programme

A BLACK sheep became a prodigal son last week, when the British Natural Environment Research Council (NERC) announced that it would be able to find the annual subscription for the new Ocean Drilling Program being organized by the National Science Foundation in the United States through the intermediary of JOIDES, the consortium of US oceanographic institutions responsible for the earlier IPOD (international phase of ocean drilling) programme. Britain participated in the planning of ODP, but did not become a full member because NERC could not pay the \$2.5 million annual subscription.

Signs that the prospects were good were seen last month, when the secretary of the council, Dr John Bowman, said that he hoped to have raised the subscription for 1986 by the end of the year. Now, it seems, the Department of Energy and other government departments have agreed to meet a fifth of the cost, with the oil companies providing 14 per cent. NERC will provide the remainder, equivalent to about £1.2 million at present exchange rates and will also stand the risk of future currency fluctuations.

Everybody seems cheerful about the prospect. At a meeting of the British coordinating committee last Friday, Dr Tim Francis of the Institute of Oceanographic Sciences was appointed as the British representative on the ODP planning committee, next due to meet at the end of January in Hawaii.

Representatives were also nominated for the dozen or so working groups of ODP, now mostly organized on regional lines, but it may be necessary to change these assignments, if, as seems likely, the structure of ODP is recast.

Even so, Britain will participate only in Leg 107 of the new programme, which is planned to sail from Malaga for several drilling programmes in the Mediterranean on 26 December next year. (The first leg of the new programme will put to sea on 31 January 1986.) There is particular British interest in Leg 14, in the Weddell Sea, in the Antarctic near the British station to be drilled in 1987.

Domestically, the British coordinating committee under Professor M.G. Audley-Charles (University College, London) will expect the British representatives on the ODP working panels to act as convenors of parallel groups among British geophysicists. The presence of the oil companies among the sponsors of the British contribution is reckoned to have been politically if not financially crucial, given the British government's belief that industrial sponsorship of research is a public good in itself. □

Japanese energy

Go-go go for nuclear power

Tokyo

JAPAN's white paper on nuclear power released this week is full of the boundless enthusiasm for nuclear energy that seems to have evaporated in the West. The call is for more nuclear power stations, the rapid development of an independent capacity to process and enrich nuclear fuel and increased effort to develop fast-breeder reactors.

The logic is simple enough. At present, nuclear power stations provide Japan with its cheapest source of electricity. According to the report, this year nuclear-generated electricity cost 13 yen (\$0.6) per kwh, against coal-generated electricity at ¥14 per kwh and liquid natural gas generated electricity at ¥17 per kwh.

The calculation for nuclear energy does not, however, include the price of decommissioning old nuclear reactors; adding this in gives nuclear power just a very slight edge over coal. Thirty-one nuclear reactors in Japan are between them supplying 23 per cent of the total electric power. That places Japan fourth after the United States, France and the Soviet Union in its total nuclear power generating capacity.

Japan's situation is, however, unique, for the nation has almost no fossil fuel reserves and must import 90 per cent of its energy, much more than any other large industrialized country. In the report's view, the cost of those fossil fuels is bound to rise over the next decade and the advantages of nuclear power become ever more apparent, although oil prices are now falling as the OPEC nations try to boost sales.

The key to Japan's cheap nuclear power is operating efficiencies that are the highest in the world. As nuclear fuel costs are small, the price of nuclear-generated electricity stems from the cost of plant construction and running and thus the percentage of time for which it is in operation. In Japan, power generation is suspended fewer than 0.1 times per year per reactor, just a tenth of the frequency of reactor troubles in the United States and attributed by plant operators to the superior training and conscientiousness of Japanese staff.

Enthusiasm for nuclear power seems to be shared by citizens too, most of whom, according to a government survey, think more electricity should be nuclear generated. Indeed, nuclear power seems to have a rather good image; when those surveyed were asked to list the uses of nuclear power, treatment of cancer was listed second after power generation.

As a government document providing a source of information for decision makers, the white paper might perhaps have painted a slightly less rosy picture. One proud boast of the government is that since commercial reactors began operation in 1966, "there have been no accidents or

failures in which operators or people living nearby were affected by radiation". That, strictly speaking, is true, but it avoids mention of the leaks on board the nuclear ship *Mutsu* and the 1981 leakage of radioactive water from a storage tank into the sea. Nor will its simple description of fast-breeder reactors as the mainstream power source of the future "fundamentally solving the problem of nuclear fuel sources" appeal to many, including those in Japan, who have tried to estimate the likely economics of these reactors.

Alun Anderson

Polish science

Congress delay

THE third congress of Polish science, scheduled for 9–10 December, has been postponed until March 1986, due to the "need for more thorough preparation of the plenary reports and also the papers being completed by the 16 problem teams". The purpose of the congress had been to review Polish science with special reference to the research applications in the economy.

The old hierarchy of graded levels of priority for research problems from "government" and "key" problems down to "departmental" problems, introduced after the second congress in 1973, has proved unworkable; some new research structure is clearly necessary. But it is apparently proving difficult to devise a viable alternative.

The first congress of Polish science (June 1951) served not only to assess the state of science but also to impose a rigidly Marxist philosophy on Polish research and learning. There are widespread fears (particularly after last month's dismissals of university administrators) that the forthcoming third congress might launch a similar purge.

Although the official reports for the congress may not be ready, one unofficial report has already appeared in the underground press. This is a 10,000 word pamphlet from the unofficial "Social Commission for Learning", which attempts to outline the effect of the crisis and martial law on Polish science and learning, including the "reorganization" of the Institute of Nuclear Research, the run-down of investment in science, the cessation of doctoral research in the universities in 1982 (it has only partially been restored), and in particular, the situation in the 200 or so research institutes responsible to some ministry or government department rather than to universities or the academy.

The pamphlet, although written in a detached and academic manner, gives a depressing picture of Polish science.

Vera Rich

AIDS

Pasteur sues over patent

Washington

THE Institut Pasteur in Paris has filed suit in the US claims court in Washington DC, contending that researchers at the US National Cancer Institute (NCI) used Pasteur's specimens and data to develop and patent a commercial test to detect antibodies to the AIDS (acquired immune deficiency syndrome) virus. The suit asks the court to declare that Pasteur researchers Luc Montagnier, Jean-Claude Chermann and François Barre-Sinoussi were the first to isolate the AIDS virus and to recognize its significance in developing blood tests for AIDS antibodies.

Institut Pasteur researchers were the first to publish an initial characterization of the AIDS virus and filed a patent application for a test in December 1983; the application remains pending. The NCI researchers, led by Robert Gallo, filed a similar application in April 1984 and received a patent 13 months later.

Montagnier has claimed that he sent his viral isolate to Gallo as a scientific courtesy for research purposes in July and September 1983. But Gallo says the rate of production of virus in the cultures he received made them useless practically, and that development of a test and reliable characterization of the virus was impossible until his laboratory found a cell line that could produce the virus in quantity.

Montagnier's original isolate has subsequently been found to be very similar to Gallo's, but Gallo angrily rejects suggestions that his isolate was in any way derived from Montagnier's. Gallo says other isolates have been found that are both more and less similar to Montagnier's than his original, and that the similarity is nothing more than might be expected given that both originated from patients in New York City.

Gallo's patent, which was turned over to the US government, forms the basis of the tests currently in use in the United States for AIDS antibodies. The commercial value of the patent is counted in millions of dollars.

Other tests are expected to enter the market shortly that are derived from the Pasteur isolates, and one manufacturer, Genetic Systems, has said it will not be paying royalties on its test to the US government.

Pasteur had been negotiating unsuccessfully with the US government for the past four months to reach a compromise agreement on royalties from existing tests, which it believes should be shared equally between the US and French institutes (see *Nature* 317, 373; 1985). It appears that the question will now be settled in court: no date for a hearing has yet been fixed.

Tim Beardsley

The cause of university education

SIR—The way in which the political ideology of a democratic government can make or mar the cause of higher education or university autonomy is best illustrated in the action of the governments of France (*Nature* 315, 172–173 & 316, 5; 1985) and Britain (*Nature* 315, 265; 1985). In France, the present government has succeeded in freeing the universities from the overbureaucratization of higher education. The granting of absolute independence to the Comité National d'Évaluation des Universités (CNEU) so that it can make an objective evaluation of a university is a step in the right direction. This may free the university from day to day interference from political or bureaucratic authority. Furthermore, CNEU wants to lay equal emphasis on research and on teaching by individuals and groups within a university. This is most certainly a radical change from current practices in French universities, where too much emphasis has been laid either on research or on teaching in granting promotion to a member of the faculty. Promotion has depended on an individual's local political influence over the central machinery responsible for promotion.

The trend in British higher education has been the opposite. The present British government has inflicted maximum damage on the cause of university autonomy, of which the British have always been proud. Much has been written on the subject in editorials in *Nature* during the past two years. *Nature* has highlighted in a systematic manner the callous disregard of the present government for the sanctity of higher education. In this connection, the address delivered by Lord Attlee on the occasion of conferment of the Honorary Degree of Doctor of Laws by the University of Glasgow in 1951 is worth quoting:

"It has always been one of our proud boasts that our universities are free and are rightly jealous of any attempt by the State to extend its power over them. The administration by the University Grants Committee is a characteristic British device which while it passes muster with the financial critics of the House of Commons, leaves the universities almost complete freedom to run their own affairs. In a democracy it is fundamental that thought should be free and that the inquiring and critical university spirit should be brought to bear on all affairs. The University must ever seek for the truth; it must never be a mere instrument in the hands of a government, a church or any political or economic group."

The respect in which the universities of Great Britain are held is mainly due to the freedom from governmental interference that they enjoy (or used to enjoy before the onslaught initiated by the present government), both constitutionally and actually.

In this crisis, the low-key position adopted by the university community is worrying. British academic institutions have almost surrendered to the political

authority in its attempt to remove the autonomy of the universities. To free us from every kind of domination except that of reason is education in the real sense. This aim can be best achieved in an atmosphere of professional integrity where teachers are as free to speak on controversial issues as any other citizen of a free society. In Cardinal Newman's words, "A university education gives a man a clear conscious view of his own opinions and judgements, a truth in developing them, an eloquence in expressing them and a force in urging them".

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PhD theses

SIR—In regard to Beverly Halstead's cogent remarks (*Nature* 316, 760; 1985) about PhD theses, Linus Pauling must have set the standard when, in 1925, he submitted the reprints of five journal articles as his thesis.

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Greenhouse dilemma

SIR—The background to the "greenhouse effect" is well-known. Carbon dioxide from burning fossil fuels, deforestation and cement manufacture causes global temperature to increase by about 3°C each time the proportion of the gas in the atmosphere doubles. At the current rate of manmade CO₂ production, the proportion will have doubled in about 230 years.

At the end of the last ice age, the global temperature increased naturally by only about 6°C with a corresponding sea-level rise of about 100 metres from melting ice in the following 2,000 years or so. By analogy, this implies a sea-level rise due to the greenhouse effect of at least 12 metres in the next 230 years with a further 38 metres still to come, even if the rate of CO₂ increase remains constant and there is no further rise thereafter. However, there is a close link between atmospheric CO₂ increase and population size.

It seems inevitable, therefore, that the greenhouse effect will induce sea-level rises high enough to drown many if not most major cities of the world and much of their agricultural hinterlands within the lifetime of the sea defences presently designed at massive cost to protect them. Alternatively, the population size might be contained and then reduced to decrease manmade CO₂ production. This would destroy the pensions and insurance structures of the developed world and family provision for the elderly of developing nations.

We appear to face a dilemma. Social structures will not allow us to reduce the population. Current technology and economics will not provide a workable alternative to the consequent continuing generation of CO₂ with its associated sea-level rise and massive incursions.

If we accept the greenhouse calculations, should we not be embracing their conclusions more urgently in the planning of relevant engineering and financial structures? On the other hand, if we choose to disbelieve the warnings, why are we paying dearly for them with public money?

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Metric system

SIR—Having grown up in the United States and lived in Britain, as well as visiting and living in several metric lands, I think A.A. Berezin (*Nature* 317, 762; 1985) has missed the point of D.C. Jolly's rather sensible letter on the metric system. A measurement system must first of all be a means by which one can orient oneself to the physical world. Any system satisfying this condition is, in that sense, as adequate as any other. I do not need to know how many square feet there are in an acre; I do need to know that an acre is a generous lot for a house but insufficient land to farm. A mile does not mean 5,280 feet to me. It means a long walk or a short drive. In my laboratory, I use the metric system exclusively, not because I believe it necessarily better but because it is the means by which I was trained to orient myself on scientific matters. When I do a physical examination of a patient, I measure and weigh in metric but report the results to the anxious human in feet and pounds. If Berezin ever told an American mother her baby had a temperature of 37 degrees, he would quickly understand my meaning. For myself as well, centigrade provides too narrow a gauge to describe bodily comfort compared to Fahrenheit, and also a fever of 104°F has a zing to it that 40°C could never match. The comforting security in tradition can sometimes more than compensate for the inconvenience of technical inefficiency.

Being universal, science needs a universal idiom and if that mandates metric, so be it. But how one sees one's personal world and describes it to others is quite another matter. For those purposes, the British-American system of measurements suits me and millions more just fine. What divides nations and peoples is not how they measure things but intolerance for the ways of others.

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Calculating the orbits of comets

The return of Halley's comet may have been a disappointment, but new-found skill at calculating cometary orbits may help towards an understanding of these objects.

THE covert return of Halley's comet has been a great public frustration that may yet send hordes of people scurrying off to the South Pacific in March and April, after the perihelion passage predicted for 9 February 1986, just six weeks from now. In reality, the likelihood that very few people would ever see the comet was well signalled in advance; the Earth is on the wrong side of its orbit to make Halley the spectacular object it seems to have been in 1066 and 1682. At a perihelion distance of 0.59 astronomical units, there can be a tenfold variation in the apparent brightness of the comet with the position of the Earth. At this unfavourable passage, many people will have to be content with reading about the event, in which case they might do worse than browse through the proceedings of last year's International Astronomical Union symposium on the dynamics of comets, which have now been published*.

Comets have an obviously special place in planetary dynamics as naturally occurring test particles in attempts at the solution of the many-body problem in celestial mechanics. Unlike the other objects in the Solar System whose mass is negligible compared with that of planets, they have the particular advantage of moving in orbits that are often far from being circular. Given the interest in spacecraft orbits and the availability of computer power, not to mention the inherent interest of the subject (and even of Halley's comet), it is inevitable that people should have been so busily calculating cometary orbits.

But is that not simply a matter of solving a few differential equations? There are two practical snags. Comets are not ideal test particles, but self-accelerating objects as a consequence of the way in which they lose mass asymmetrically near perihelion. And even the best-known, among which Halley and Encke (found at the end of the eighteenth century) stand out, travel in orbits whose elements are poorly known, at least in comparison with the orbits of the planets.

Running through last year's symposium seems to have been a hymn of gratitude to B.G. Marsden from the Harvard-Smithsonian Institute of Astrophysics for his compilation over many years of an authoritative list of the orbital elements of more than a hundred periodic comets and for having, during the past two decades, founded the small but flourishing school of those who would put the treatment of

the "non-gravitational" influences on cometary orbits on a rational basis.

What began as an empirical attempt to calculate the acceleration (sometimes the deceleration) of the comet from the changes in its orbit, so as to learn something of the physics of the processes responsible, has been rationalized by supposing extra forces which are functions of the heliocentric distance. On this view, comets accelerate by the transfer of momentum from the escaping gases; for the case of Encke, Y.V. Batrakov and Y.A. Chernetenko from the Institute of Theoretical Astronomy at Leningrad now think that the non-gravitational forces can be related to the magnetic index of solar activity, which makes sense.

In practice, the outcome of these calculations seems as satisfactory as could be expected. The assumption that the non-gravitational force on a comet near perihelion is simply a function of the heliocentric distance and, specifically, that it is a power law works well enough. But the idea that the force could be in any direction than that towards the Sun requires that the nucleus of the comet should be spinning, whence the hope that the Giotto encounter next March may directly throw light on the revolution of the Halley nucleus.

Even without these qualifications, the solution of the differential equations of the motion of a comet is far from being simple. One obvious difficulty is that even the comets with very short periods are known accurately only from their brief passage through perihelion, so that integration entails projecting a set of non-linear equations forward for the equivalent of many years from a poorly known starting point. So far as can be told from last year's meeting, computers have made possible several new ways of tackling the problem, which is why it is (or should be) all the more humbling that, at the very beginning of the eighteenth century, Halley had applied methods to the calculation of twenty cometary orbits and that, by the middle of the century, A.C. Clairaut had been able to allow for the effects of Saturn and Jupiter on the orbit of Halley's comet so as to predict its passage in 1757 to within a month.

The neatest, or at least the most ambitious of all the programmes of calculation described last year, is that which yields comet orbits as by-products of a continuing international project to calculate

the evolution of the Solar System as a whole, known as the Long-Term Evolution Project. The project has the virtue of involving not merely people from Western Europe (among whom Italians are prominent), but also at least one group from Czechoslovakia. The objective is that the motion of the principal objects of the Solar System should be calculated almost from first principles. As with other numerical schemes of this kind, among which that of Edgar Everhart from the University of Denver is the best known, the proof of the pudding consists not of the agreement between predictions and future observations, but of agreement between the calculations and past records of the passage of known comets — "postdiction" as it is anachronistically called.

Many of the results really are quite startling, familiar though they may be to those working in the field. Even Halley is not as well-behaved as might be thought. D.K. Yeomans of the Jet Propulsion Laboratory describes the attempts made since Halley's own work in 1705, including that of Bessel in the early nineteenth century and of those this century who have had the benefit of the ancient Chinese records, only to conclude that this long series of perihelia, if used as the basis of strictly dynamical interpolation, foreshortens each cycle of roughly 76 years by about four days. With the discrepancy explained by the transfer of momentum at evaporation of the surface material, Yeomans concludes that the orbit of Halley's comet can have changed only slowly during the past 18,000 years or so, but that there may have been some more profound interaction with Jupiter at some point.

This fits in well with the calculations made of the behaviour of other comets in the past. There is for example, the case of the comet Oterma, whose orbit lies between those of Jupiter and Saturn, which encountered Saturn in the late eighteenth century, which has just spent two decades as a distant satellite of Jupiter, but which is now likely to return to much what its former orbit was. This is one of many examples quoted at the symposium to illustrate how comets filter their way from the supposed Oort cloud of comets-in-waiting, at some distance beyond Neptune, by successive encounters with planets into the inner Solar System. The comets we see are the exceptions.

John Maddox

**Dynamics of Comets: Their Origin and Evolution* (eds Carusi, A. & Valsecchi, G.B.) (Reidel, Dordrecht, 1985).

Membrane proteins

Structure of a bacterial photosynthetic reaction centre

from Richard Henderson

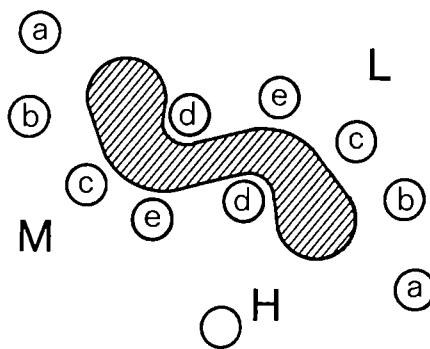
ON PAGE 618 of this issue J. Deisenhofer *et al.* report the first structure at atomic resolution of a protein — or, more accurately, a complex of several proteins — from a biological membrane. Not only is it a superb structure but since it is of a photosynthetic reaction centre complex (from the purple bacterium, *Rhodospseudomonas viridis*), the result provides a structural basis for understanding photosynthesis. In addition, the work bears witness to the tremendous and continuously growing power of protein crystallography.

A great handicap in understanding any of the vital processes carried out by membrane proteins has been the lack of atomic structures for such proteins. The major problem is to release the proteins in undamaged and stable form from the lipid bilayers that constitute the membranes. There is no such problem with the water-soluble cytoplasmic proteins, 200 or so of which have had their structures solved. Ironically, since water-soluble proteins need a non-polar interior and a polar surface whereas lipid-soluble proteins could be largely hydrophobic and their internal hydrogen bonds would not have to compete with the hydrogen-bonding potential of the surrounding water, the architecture of membrane proteins might actually be simpler than that of cytoplasmic proteins.

It was over three years ago that Hartmut Michel produced large well-ordered crystals on the *Rps. viridis* reaction-centre complex using a method somewhat similar to those used to crystallize many water-soluble proteins, but based on a thorough understanding of the behaviour of the protein-detergent complex in the presence of small amphiphiles and of the precipitant, 2.5 M ammonium sulphate (*J. molec. Biol.* **158**, 567; 1982). There then began a fruitful collaboration between Michel and one of the most productive protein-structure groups in the world, headed by R. Huber. The crystals turned out to contain only one molecule of the complex per asymmetric unit, surrounded by solvent that fills more than 70 per cent of the crystal volume. After collection in a darkened cold room of X-ray diffraction data from the native protein and several carefully selected heavy-atom derivatives, an initial map of the structure at 3 Å resolution was obtained in which the chromophores were immediately identifiable (Deisenhofer, J. *et al. J. molec. Biol.* **180**, 385; 1984).

Further interpretation of the electron-density map has now allowed all the components of the photochemical reaction centre to be precisely located. The centre

contains four molecules of bacteriochlorophyll-b (BChl), two of bacteriopheophytin-b (BPh), two different quinones, a non-haem iron and four haems, all embedded in a protein that is made up of four polypeptide subunits containing a total of 1,187 amino-acid residues. The crystallographic unit cell of the reaction centre is smaller than that of the icosahedral viruses whose structures have re-



Schematic diagram illustrating a cross-section through the photosynthetic reaction centre of *Rp. viridis* showing the relationship between the 11 trans-membrane helices (5 of subunit M, 5 of L and 1 of H) and the region occupied by the chromophores, which is shown shaded. The helices are all tilted by angles of up to 38° to the direction of view, which is from the periplasmic side. They are therefore much closer together at the cytoplasmic side of the membrane and much further apart at the periplasmic surface than shown here, which is at a level close to the centre of the membrane.

cently been solved (see Harrison, S.C. *Nature News and Views* **317**, 382; 1985), but unlike them it is devoid of symmetry. It is the largest and most complex atomic structure yet determined. It is even more remarkable that the heart of bacterial photosynthesis, which is similar to photosystem II of green plants, should be the first membrane structure to be solved in detail.

Chromophores

All the chromophores in the complex have been precisely located. The quinone sites include both the strongly bound menaquinone (Q_A) which is found in the native structure, and the more weakly bound ubiquinone (Q_B), which was initially absent in the crystals and had to be located by the difference Fourier method after soaking the crystals in solutions of quinones and quinone analogues. The structure and function of the chromophores in the complex have been reviewed by J. Barber (*Nature News and Views* **315**, 278; 1985). All the chromophores except the haem groups of the

cytochrome are related in pairs by a local 2-fold axis which is perpendicular to the plane of the membrane. Thus on the periplasmic side of the membrane, a pair of BChl molecules are found in close contact with one another. They are called the 'special pair' ($BChl_2$), because spectroscopy has shown that they are in contact and that their oxidation is the primary event in photosynthesis.

Two alternative paths of electronically conducting chromophores extend from this special pair to the single non-haem iron atom which is positioned on the opposite (cytoplasmic) side of the membrane: each path consists of the sequence $BChl_2$ -BChl-BPhe- Q_A or Q_B -Fe. These chromophores accept excitation energy from light-collecting complexes of protein and chlorophyll and convert it rapidly into a charge separation across the full width of the membrane — approximately 40 Å. An electron is transferred from the (oxidized) $BChl_2$ to Q_B within 230 picoseconds, apparently always via Q_A which, after reduction, transfers an electron to Q_B , possibly via the non-haem iron. (It is not clear why electron transfer does not also proceed along the other pathway, directly to Q_B .) Following charge separation in the membrane, the $BChl_2$ is re-reduced by the cytochrome at the periplasmic surface, while Q_B , when fully reduced, is protonated at the cytoplasmic surface and released into the pool of free quinone in the bilayer.

Protein

The four polypeptides — L, M, H and a c-type cytochrome — envelop the chromophores. The L and M subunits provide the body of the structure within the membrane. Each forms an open crescent consisting of a curved wall of five transmembrane helices (a–e in the figure). The chromophores are not totally surrounded, so the L–M protein dimer cannot be described as globular. Indeed, since the chromophores are themselves hydrophobic they need not be separated from a lipid environment. Furthermore, BChl must receive energy from BChl in the light-collecting complexes which sit nearby, within the lipid bilayer, and Q_B must exchange with the pool of free quinone in the bilayer. Thus the function may require direct access to the chromophores from the bilayer. For these reasons, the arrangement of hydrophobic protein helices, hydrophobic chromophores and hydrophobic lipid may be rather open.

The transmembrane helices themselves may have three functions. They may surround the chromophores with non-polar side chains designed to hold them in precise relative orientations. In addition, they contribute the single histidines linking the Mg^{2+} of each half of the $BChl_2$ to the protein and the four histidines coordinated to the non-haem iron; two other histidine ligands to BChl and a glutamic acid ligand to the iron are in non-helical

segments of subunits L and M. And they provide an energetically stable conformation for the polypeptide and a smooth exterior facing the bilayer.

Outside the membrane on the periplasmic surface lies the *c*-type cytochrome with its four haem groups arranged in tandem, allowing electrons to jump from one to the other, ready to re-reduce the special pair of BChl. On the cytoplasmic side sits subunit H, of unknown function, but perhaps involved in some way with the function of quinone Q_B and the determination of the mode of interaction with the light-collecting complexes. Both the cytochrome and the H subunit are globular proteins which, although unique in their fold, have structures typical of many water-soluble globular proteins. The H subunit has an amino-terminal hydrophobic tail which forms a single transmembrane helix, much as is presumed to occur in proteins such as glycophorin.

The structure thus provides a detailed and precise three-dimensional model for the events of photochemical energy conversion. It confirms many previous hypotheses based on other methods and allows new propositions to be formulated. For example, since the weak sequence homology between subunits L and M and the proteins D1 and D2 from photosystem II of higher plants is much more convincing when critical residues in the *Rps. viridis* structure are examined, it is now postulated that D1 is homologous to L, and D2 to M, and that D1 and D2 surround the equivalent of BChl₂ in photosystem II of higher plants.

Construction of the X-ray model depended on sequences of the *Rps. viridis* proteins determined by Michel and his colleagues. But the distribution of hydrophobicity in the amino-acid sequences of the same polypeptides in related bacteria had already led to the prediction of a structure containing five transmembrane helices in each of the L and M subunits and one in the H subunit of *Rps. capsulata* and *spheroides*. The qualitative success of these predictions makes one confident that the many other current predictions of the position of helices in membrane-crossing proteins will turn out to be correct, even if they can be made only in broad one-dimensional terms. It now seems likely that α -helices spanning the membrane will form the hydrophobic domains of all membrane proteins and membrane-protein complexes, with the possible exception of those that form very large aqueous channels, where other secondary structures might be preferred.

The open arrangement of the membrane-spanning α -helices in the reaction centre is different from the more tightly packed helices in bacteriorhodopsin (Henderson, R. & Unwin, N. *Nature* 257, 28; 1975), where they have to line a rather more hydrophilic proton channel, and very different from the arrangement in the porins of the outer membrane of *Escher-*

ichia coli, where β -sheets surround an even larger hydrophilic pore (Kleffel, B. *et al. EMBO J.* 4, 1589; 1985).

When more details of the structure become known, it will be especially interesting to see how the redox potentials of the different chromophores and the transfer of electrons between them are regulated by the stereochemistry of their interac-

tions with the surrounding protein. For now, there is much pleasure to be gained simply from contemplating the superb design of a protein that has its body immersed in a lipid bilayer and its head and legs in water. □

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Planetary science

Hidden carbon dioxide on Mars

from Robert M. Haberle

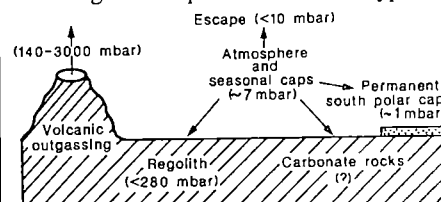
THE atmospheres of Venus and Earth are the primary reservoirs for their major constituents: virtually all of Venus's outgassed carbon dioxide is in its atmosphere, and the same is true for nitrogen on Earth. Mars, however, is different. The amount of carbon dioxide in the martian atmosphere, about 7 mbar, is much less than the planet was originally endowed with, and researchers attempting to sort out the climatic history of Mars are keenly interested to know the fate of the rest. In a recent paper, R. Kahn¹ suggests that much of the outgassed carbon dioxide on Mars is tied up in its crust, in the form of carbonate minerals.

It has long been recognized that the carbon dioxide released into the martian atmosphere may have been incorporated into carbonates². If the total amount of carbon dioxide vented into the martian atmosphere were at the high end of the range of estimates (140–3,000 mbar¹), the idea of a carbonate reservoir becomes particularly attractive for several reasons. First, the polar caps and the regolith, the loosely consolidated rocky surface, which are potentially the largest reservoirs for carbon dioxide other than carbonates, do not seem to have the capacity to hold more than the equivalent of about 300 mbar, so that other sinks are required (see the figure). Second, if Mars degassed large amounts of carbon dioxide early in its history, which is consistent with models of the observed nitrogen isotope ratio³, a significant greenhouse warming would have resulted in surface temperatures that could have been high enough to permit liquid water. This is a leading explanation of the 'runoff' channels found throughout the old cratered uplands in the southern hemisphere⁴. In the presence of liquid water, 1 bar of carbon dioxide could be converted into carbonate rocks in the order of 10^7 years⁵.

Kahn's new suggestion¹ is that carbonate formation on Mars continued after open bodies of liquid water became unstable. With an adequate heat source and sufficient quantities of ice, Kahn argues that transitory pockets of liquid water in disequilibrium with their environment could form; in their presence, carbon dioxide would continue to be converted

into carbonates.

A key quantity in Kahn's hypothesis is the minimum atmospheric overburden pressure, P^* , required for liquid water to form. Below that minimum value, ice would sublime before melting. A reasonable lower limit of P^* is 6.1 mbar, the triple point of water, because with atmospheric pressures below the triple point, liquid water would rapidly sublime. An upper limit is much more difficult to determine but may be as high as 30 mbar, though Kahn gives no detailed calculations to support this value. The most interesting consequence of his hypoth-



Possible reservoirs for carbon dioxide on Mars. Of the 140–3000 mbar of carbon dioxide estimated to have been vented into the martian atmosphere, less than 20 mbar can be accounted for in the atmosphere and polar caps, and lost into space. The two most likely reservoirs for the remainder are the regolith, a near-surface layer of pulverized soil created by meteoritic bombardment, and carbonate rocks, a reservoir created by weathering processes.

esis is that, in the absence of a recycling mechanism for carbon dioxide, the surface pressure of Mars will monotonically decline until it reaches P^* , whatever its value. Kahn believes that the surface pressure observed is very close to P^* .

Before the Viking mission, the surface pressure on Mars was believed to be controlled by the temperature of the permanent polar caps⁶. As the caps represent the solid phase of the atmosphere, the annual mean surface pressure must be equal to the saturation vapour pressure corresponding to their average temperature. But Viking found no evidence for substantial permanent polar caps: the carbon-dioxide frost deposits at the north pole completely disappear during northern summer and the amount that remains at the south pole during southern summer is too small to buffer global pressures, and may even disappear completely in some summers⁷. Thus, it seems unlikely that the

polar caps buffer atmospheric pressure.

Kahn's theory has another implication, apart from the explanation of the low surface pressure. If he is correct, the climate of Mars has evolved linearly over geological time, rather than cyclically. This represents a major challenge to current theories of martian climatic history, which generally invoke a periodic exchange of carbon dioxide between reservoirs in the regolith-atmosphere-cap system to explain the polar layered terrains^{2,3}. The exchange, driven by variations in the orbital parameters of Mars, can result in surface pressures that vary from 0.1 mbar at low obliquity (11.7°), to about 15 mbar at high obliquity (37.1°). These variations are thought to modulate the transport and deposition of dust and water into polar regions, so giving rise to the layered terrains. Over many obliquity cycles, the mechanism would substantially deplete the regolith reservoir and eliminate cyclical climatic changes, because conversion of carbon dioxide into carbonates on Mars is irreversible.

Not surprisingly, there are many uncertainties over the plausibility of Kahn's mechanism. Among them are availability of cations, such as Mg²⁺ and Ca²⁺, for carbonate chemistry; the dependence of P^* on heating and soil properties; and whether or not the favoured regions for carbonate formation could be resupplied with water. It would also be worth knowing just how much liquid water is required. Furthermore, no signature of the vast amount of carbonate that must have been produced has been unequivocally identified. Kahn's discussion of these points is mostly qualitative; the basic physics of the process, therefore, remains to be demonstrated. Nevertheless, it is an intriguing idea that merits serious attention. □

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Geophysics

Transient mantle rheology

from J. Weertman

CONSIDERATION of the ways in which solids can deform leads to the conclusion that the viscosity of the Earth's mantle should increase with depth. This agrees with our current ideas about the thermal structure of the interior of the Earth. Hence it came as a surprise to find that analyses¹⁻³ of glacial rebound and complementary Earth satellite data indicate that the mantle viscosity does not increase with depth but is almost a constant, with a value of about 10^{21} Pa s. Until now, this has presented a dilemma to those studying the physical structure of the Earth and its consequences for plate tectonics but, in this issue of *Nature*, W.R. Peltier⁴ shows that the problem can be resolved if transient creep is taken into account.

The modern study of the creep deformation of the Earth's mantle started with the suggestion⁵ that the mantle might deform plastically through the diffusional transport of atoms between grain boundaries, the mechanism now known as Nabarro-Herring creep^{6,7}. Using the Nabarro-Herring creep theory, R.B. Gordon⁸ found that the mantle viscosity increases with depth. His result is readily understood. The greater the difference between the actual temperature at a given depth and that at which mantle rock first starts to melt, the larger the expected value of the viscosity. The further rock is from its melting temperature the 'colder' it is (even if its actual temperature is quite high) and the more difficult it is to make the rock creep. As the mantle convects, its thermal profile should be approximately

adiabatic. The adiabatic temperature presumably increases much less rapidly with depth than does the temperature at which melting commences and hence it would not be surprising to find that viscosity increases with depth in the mantle.

This conclusion is not dependent on the choice of the creep mechanism which is used to obtain an estimate of the mantle viscosity. Any such mechanism gives faster creep the closer the temperature is to the melting temperature. It also is not dependent on whether the creep mechanism gives rise to a linear, newtonian creep equation ($\dot{\epsilon} = \sigma/\nu$) or to a non-newtonian creep equation such as a power-law creep equation ($\dot{\epsilon} = c\sigma^n$). Here $\dot{\epsilon}$ is the creep rate, σ is the stress, ν is the viscosity and c and n are constants. (For non-newtonian creep an effective viscosity is defined to be equal to $\nu = \sigma/\dot{\epsilon}$. The effective viscosity for non-linear creep requires that a standard stress level or strain rate be specified.)

That the mantle viscosity is almost constant could be explained, of course, if the ratio of the actual temperature over the melting temperature were constant throughout the mantle. It is more likely, however, that this ratio decreases with depth. It could also be explained if mantle creep obeys a power law⁹ and the creep stress increases with depth at a suitable rate. But the glacial rebound stresses decrease with depth. One possible way out of the dilemma is to bring transient creep phenomena into the analysis. In analyses carried out up to now it has been assumed implicitly that only steady-state creep

equations need be considered.

Analyses of isostatic geoid anomalies^{9,10} indicate that the lower mantle has viscosity an order of magnitude greater than that of the upper mantle and hence than the lower mantle viscosity as determined from glacial rebound. But geoid isostasy operates on a timescale of the order of 10^6 years rather than the 10^4 – 10^5 years for glacial rebound. Peltier⁴ investigates whether the order of magnitude difference of viscosity of the lower mantle as deduced from the two phenomena could arise if the glacial rebound method measures a predominantly transient creep strain rate and if the isostatic geoid anomaly method measures a more steady-state creep strain rate.

Peltier makes an explicit calculation by use of a linear creep equation that reduces to a steady-state equation at long times but is a transient equation at shorter times. This equation is for a Burgers's solid¹¹ which, when springs and dashpots model the solid, is simply a Zener standard linear solid in series with a dashpot. For uniaxial stress-strain the equation he used can be written as $\dot{\epsilon} + (\sigma/\tau) + (\mu^*\sigma/\nu\tau) = \mu\dot{\epsilon} + \mu^*(\dot{\epsilon}/\tau)$ where the dot stands for time differential, τ is a decay time, μ and μ^* are elastic moduli and ν is the long-term viscosity. Peltier uses this equation only for the lower mantle. For the upper mantle he uses a Maxwell solid (spring and dashpot in series) with the equation $\dot{\epsilon} = (\dot{\sigma}/\mu) + (\sigma/\nu)$. He is able to show that it is possible to reconcile the two values of the viscosity of the lower mantle. He points out that further constraints on the transient creep contribution to lower mantle deformation may require reexamination of the analysis of Richards and Hager¹⁰.

Since Peltier's paper was submitted for publication, a paper by Sabadini, Yeun and Gasperini¹² has appeared in which a transient creep analysis also based on a Burger's solid has been carried out. The results and conclusions are very similar to those of Peltier. Clearly, the study of the rheology of the mantle is entering a new period in which many possible creep mechanisms are being considered as rate controlling, and in which the theorists have become capable of carrying out quite sophisticated studies involving transient creep of mantle rocks. □

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Plant tumours

Wounds activate attackers

from John E. Beringer and Colin M. Lazarus

THE ability of strains of *Agrobacterium* to induce tumours on dicotyledonous plants has excited the interest of most biologists, particularly since it was discovered that tumour formation results from the inheritance of DNA (T-DNA) transferred from the bacterium to the plant, a process that depends on the function of virulence (*vir*) genes. Tumours are induced only where there is a wound on the plant and an intriguing question has been how *vir* genes are regulated so that *Agrobacterium* can recognize wounds and initiate T-DNA transfer. The answer, provided by S.E. Stachel *et al.* on page 624 of this issue, is surprisingly simple: the *vir* genes are induced by specific signal molecules, acetosyringone and α -hydroxy-acetosyringone, that are produced by wounded, but not by intact, plant cells.

This finding helps us to understand why wounds are required and demonstrates yet again how much tumour formation depends on interactions between the two partners. A series of interactions can now be recognized that are logical and all, apparently, directed towards optimizing the efficiency of the interaction from the point of view of the bacterium.

T-DNA is present as part of a large extrachromosomal molecule (plasmid) that also carries a number of *vir* genes. Like other DNA elements capable of transposition, T-DNA is bounded by identical repeats of DNA. Independent loops of T-DNA are found when *Agrobacterium* is cultured in the presence of plant cells and it is assumed that these are T-DNA intermediates in the transfer to the plant cells. Seven genes are required for T-DNA transfer. Two of them, *virA* and *G*, are expressed in vegetative cells but the other five are induced only in the presence of acetosyringone or α -hydroxy-acetosyringone.

Once it is transferred to the plant, T-DNA integrates into host DNA and the genes it carries are expressed. T-DNA encodes genes involved in the synthesis of plant hormones and for the synthesis of novel compounds (opines) that are produced only by plant cells containing T-DNA from *Agrobacterium*. Tumour formation appears to be a method evolved by *Agrobacterium* for inducing the plant to produce a large number of cells that synthesize opines, which are metabolized by *Agrobacterium* but by few other organisms.

Opines have also been shown to act as specific inducers of the transfer (*tra*) genes carried on *Agrobacterium* tumour-inducing (Ti) plasmids. It is now clear that plant metabolites induce the functioning of two distinct sets of *Agrobacterium*

genes: those for virulence and those for transfer of Ti plasmids. In both cases, DNA replication and mobilization leading to gene transfer are induced only when they are likely to be of direct value to *Agrobacterium*.

As well as answering the immediate questions of why only wounded tissue is infected by T-DNA, Stachel *et al.* may also have resolved the enigma of why strains of *Agrobacterium* carrying *Rhizobium* nodulation genes form nodules on the roots of appropriate legumes, whereas they induce tumours when inoculated on wounded tissue; presumably the close association of the bacteria and plant cells during nodule development does not lead to the induction of *vir* genes because the plant is not wounded.

This report is another example of how the study of plant-microbe interactions can yield important information on genetic regulation. Building on evidence that plant defences against pathogenic fungal attack are triggered by fragments of the host plant cell wall, K. Tran Thanh Van *et al.* (*Nature* 314, 615; 1985) have recently

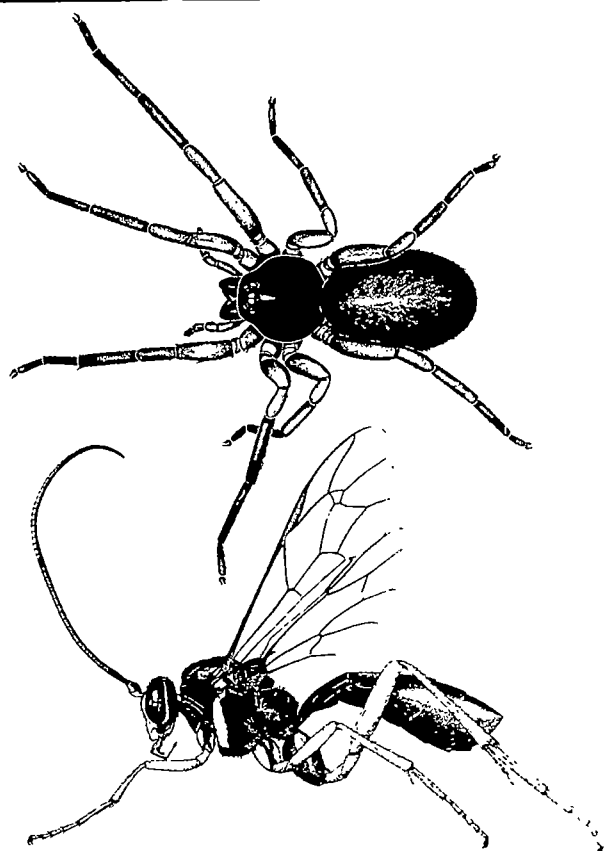
shown that specific oligosaccharides can affect the developmental fate of plant cells in culture. This indicates a role for such 'oligosaccharins' in normal organogenesis. Recently J.T. Mulligan and S.R. Long (*Proc. natn. Acad. Sci. U.S.A.* 82, 6609; 1985) and L. Rossen, C.A. Schearman, A.W.B. Johnston, and J.A. Downie (*EMBO J.*, in the press) have reported that a *Rhizobium* nodulation gene is induced by root exudates in an analogous manner to *Agrobacterium vir* genes. These findings identify primary signalling molecules and raise questions concerning the transduction of signals from the cell surface to the genetic material.

What about interactions such as those of mycorrhizas and between host plants and other pathogens? Progress

in the search for similar inducers may be slow. As Stachel *et al.* have shown, finding an effect of root exudates is only the beginning of a complex isolation and characterization process. To purify the molecules responsible it is necessary to have a good assay, which in general is not an assay of a plant response. Both Mulligan and Long, and Stachel *et al.* took advantage of the extensive genetic understanding of *nod* and *vir* genes, respectively, to produce fusions of these genes with the *Escherichia coli lacZ* gene and thus could determine induction by measuring β -galactosidase activity using a simple and sensitive assay procedure.

We look forward to seeing a surge of activity in the identification of plant molecules that act as specific inducers of genes in symbiotic and pathogenic microorganisms. Perhaps, as with other aspects of plant-microbe interactions, work will be driven by studies of the genetically well-characterized bacteria *Agrobacterium* and *Rhizobium*. It is good to see that studying the biology of tumour formation can yield results that are at least as satisfying as those obtained through molecular studies of DNA.

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The spider, *Subantarctia* sp., forms the frontispiece to the first formal presentation of the *Orsolobus* taxon of spiders, members of which are found in South America, Australia and New Zealand (Forster, R.R. & Platnick, N.I. *Bull. Am. Mus. nat. Hist.* 181, 1; 1985). The fly is the frontispiece to a taxonomic study of the ichneumon-fly genus *Banchus* in the Old World (Fitton, M.G. *Bull. Br. Mus. nat. Hist.* 51, 1; 1985).

Biophysics

XANES spectroscopy and the crystallographic imperative

from John Galloway

PERHAPS nowhere in the determination of molecular structure is precision more at a premium than in the case of metalloproteins, so that any method that promises to enhance or improve on single crystal protein crystallography is worth investigating. X-ray near-edge structure (XANES) spectroscopy is claimed to represent such promise. The real question, of course, is whether that promise can be realized; in the paper by A. Bianconi *et al.* on page 685 of this issue we might have had the first chance, to find out. As it happens, we don't.

What exactly is XANES? Absorption of X-rays takes place chiefly through the photoelectric effect. An electron is ejected from the K shell of an atom (or possibly some other shell). Its chances of escaping are modulated by its being scattered by neighbouring atoms. The resulting fine structure in the absorption cross-section expressed as a function of the energy of the incident X-ray photon contains information about the local atomic geometry. For energies not much in excess of the threshold represented by the absorption edge, say within 50 eV, the photoelectrons are strongly and multiply scattered. These modulations are referred to as XANES.

At higher energies the scattering is weaker and dominated by single scattering events. In this energy regime the fine structure is the rather better known extended X-ray absorption fine structure (EXAFS), which has been widely used in various types of structural study, including some on metalloproteins, since about 1972, the year in which it first became possible to replace the conventional X-ray tube by synchrotron radiation. The domination by single scattering events confers on EXAFS an immediate advantage it shares with a number of other physical probes of structure, namely that it is relatively easily interpreted. But it also confers the considerable disadvantage that it contains information only about two-particle correlation functions, so that only the *radii* of the coordinating shell of atoms and slightly more remote shells in metalloproteins can be extracted.

XANES, by virtue of its strong multiple scattering, contains information about three- and higher-order correlations. Thus, in principle, information about the detailed geometry of arrangements in the shells should be extractable — angles as well as distances. A lot is made of this feature by Bianconi *et al.* The Fe–C–O bond angle is obtained in a single crystal of carboxymyoglobin from the angular de-

pendence of the XANES spectrum, and a difference in this angle between carboxymyoglobin and carboxyhaemoglobin is shown to exist in solution. Unfortunately, the paper gives no indication of the accuracy of these measurements although it is unlikely to be better than $\pm 10^\circ$. On the face of it this seems to compare quite well with the angular accuracy that can be achieved by conventional crystallography but the question is not explicitly addressed by the authors.

That XANES might be interesting at all to structural biologists is a consequence of the two cardinal deficiencies of single crystal methods. First, that they are obviously only suited to crystals, whereas XANES and EXAFS can and do provide structural information about proteins in solution. Second, that in single crystal determinations the extent and quality of the data limit accuracy; limited resolution and phasing errors typically lead to a precision in atomic position of no better than 0.3–0.5 Å, although with great care standard deviations on atomic positions can be brought well below 0.1 Å, which will be needed for understanding function in metalloproteins, where distances between metal ions and their ligands may need to be known to better than 0.1 Å resolution.

Without a good idea of a method's accuracy, realized or potential, no technique will or should be taken seriously. Early proponents of EXAFS rashly made unfounded claims of its superiority over crystallographic analysis of haemoglobin (Eisenberger, J.B. *et al.* *Nature* **274**, 30; 1978) and got a bloody nose for their pains (Perutz, M. *et al.* *Nature* **295**, 535; 1982). But EXAFS has been rehabilitated

through very careful comparisons with crystallographic analysis on 2Zn insulin (Bordas, J. *et al.* *Proc. R. Soc. B* **219**, 21; 1983) and on haemoglobin and insulin (Dodson, G. *Proc. Bioinorganic Discussion Group*, Daresbury, in the press). Dodson concludes that EXAFS has a "clear role in adding precision to the crystallographic picture and hence furthering our insight into the chemical and structural processes that are at the centre of biological phenomena".

Whether the same is true of XANES will be found only in much the same way and will not be easy. It is crucial that the early mistakes made with EXAFS are not repeated with XANES, necessitating rescue operations at a later date. Systematic comparative structural studies of XANES and conventional crystallographic analysis now will pay dividends. The advantage of crystallographic analysis is that production of the electron-density map is not subject to the experimenter's anticipation. XANES is model-dependent — a structure must be imagined and its consequences compared with the experimental data. So the only sound tactic is a heavy investment of effort in alternative structures — a prominent feature of the papers of both Bordas *et al.* and Dodson.

XANES analysis tends to be very heavy on the time of both experimenters and computers. Furthermore, it has inherent limitations, as pointed out by J. B. Pendry (*Comments Solid State Phys.* **10**, 219; 1983). The range of data is small and will not generally be expected to contain many features to be fitted to the model. Indeed, it seems highly optimistic to suppose that the positions of more than three atoms will be found with any accuracy. Therefore, it will not be useful except in harness with other methods and even then its usefulness is likely to lie in choosing between qualitatively different structural alternatives where much is already known. □

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Ecology

Rats as agents of extermination

from Jared Diamond

"TAKE care! Kingdoms are destroyed by bandits, houses by rats, and widows by suitors!" Biologists would emend this insight of the 17th century Japanese poet Ihara Saikaku to note that rats destroy not only houses but also island biotas. The colonization of islands by rats has outweighed all other causes of extinctions of island birds. Rats have also preyed on young Galapagos giant tortoises and exterminated small mammals, large insects, molluscs (Hawaiian achatinellid land snails), and cold-blooded vertebrates (most ground snakes and lizards of Mauri-

tius plus mainland frogs, lizards and tuataras of New Zealand).

Yet it has proved hard to make sense of these gruesome facts. Rats have caused catastrophic extinction waves on some islands, a few extinctions on others, and no visible effect on still others. Somehow, these varying outcomes must depend on the differing susceptibilities of prey species, and on the differing biologies and histories of the three species of rats involved. Ian Atkinson of the New Zealand Department of Scientific and Industrial Research has now made a major contribu-

tion to sorting out this confused situation (in *Conservation of Island Birds* ed. Moors, P.J., 35; International Council for Bird Preservation, Cambridge, 1985). By scrutinizing historical records for the past several thousand years, Atkinson has been able to reconstruct the spread of each rat species. He has thereby established both how the impact of each species differs and the variation in the susceptibility of different faunas exposed to the same rat species. Atkinson's conclusions are not only important to conservation biologists but have also yielded an unexpected insight into the evolution of predator avoidance behaviour.

The three main commensal rat species are the Polynesian rat *Rattus exulans* (here abbreviated *RE*) originally native to tropical southeast Asia, the ship or black rat *R. rattus* (*RR*), from India and southeast Asia, and the Norway or brown rat *R. norvegicus* (*RN*), originally from Siberia and China. These rats reach islands as stow-aways on ships. The first expansion (1500 BC–AD 1000) was of *RE*, which reached Pacific islands on Polynesian canoes. From AD 1500–1700 *RR*, by then established in Europe, was spread by ship to islands of the Atlantic and Indian Oceans. By 1716, *RN* had largely displaced *RR* in Europe and was the main rat to ride ships to islands, including some in the Pacific. After 1850, for unknown reasons, *RR* again became the dominant shipboard rat. The spread of rats to islands has proceeded at a fairly steady rate from 1850 to the present, with a peak during and after the Second World War related to establishment of military bases on islands.

With this knowledge of what islands acquired which rats and when, Atkinson then identified the impact of each species. *RE* can climb trees but is the smallest of the three rats and, in terms of documented victims, seems to prey on the fewest species of birds. It may, however, have claimed many undocumented and long extinct species on the Pacific islands during its expansion before AD 1000, and it does still limit the distribution of some invertebrates and cold-blooded vertebrates. (Ironically, the largest bird known to be killed by any rat, the Laysan albatross *Diomedea immutabilis*, is a victim of the smallest rats *RE* — see figure). The largest species, *RN*, climbs trees infrequently and has mainly devastated ground-nesting or burrow-nesting birds, especially seabirds. *RR* is medium-sized but an agile climber and preys on birds at any height from the ground to the canopy. Rats can also have an indirect effect on island biotas by acting as prey for predatory cats, weasels and mongooses, which might otherwise not survive and which also prey on island native species.

In three or four notorious cases, invasions of formerly rat-free islands by rats have rapidly been followed by biological catastrophes. For example, three years after *RR* reached Big South Cape Island (off



Pacific rat approaching (inset) and eating a Laysan albatross. (Photo courtesy Cameron Kepler.)

New Zealand) in 1962, it had reduced or exterminated over 40 per cent of the island's landbird species plus the last surviving population of the southern short-tailed bat. *RR* caused similar catastrophes on Lord Howe Island in 1918, Midway in 1943, and probably Hawaii in the late 1800s. On the other hand, numerous islands with endemic native rats have survived the arrival of *RR* (and sometimes also *RN*) without any subsequent extinctions of birds. This was the case, for example, in the islands of Galapagos, Christmas (Indian Ocean), West Indies, Andaman, Nicobar, New Guinea and the Solomons.

This contrast suggests that birds that have co-evolved with native rats develop predator defence behaviours that allow them to survive invading rats. This, however, is not the sole or even main reason why islands biotas differ in their susceptibility to rats. Many initially rat-free tropical islands (Fiji, Samoa, Tonga, Marquesas, Aldabra and others) have survived colonization by rats without catastrophe. Atkinson notes that land crabs, which are widely distributed on tropical islands, are not only scavengers and grazers, as usually assumed, but also eat birds' eggs and chicks; moreover, some species (especially the giant coconut crab *Birgus latro* and some hermit crabs) climb trees. Thus, they are the invertebrate equivalents of rats, and co-evolution of birds with land crabs may lead to the development of anti-predator behaviour that also protects the birds against invading rats.

Atkinson's analysis has identified new questions. Why did *RR* partly displace the larger *RN* on ships after 1850? Will archaeologists in Polynesia document waves of extinction attributable to the arrival of *RE* with the first Polynesians? What was the original distribution of land crabs? What, specifically, are the predator defense behaviours that are produced by co-evolution with rats, and perhaps land

crabs?

But the most important problem is a practical one. Gough, Inaccessible, Nightingale, Clipperton, Snares, Laysan, and some Galapagos islands, to name just a few, remain rat-free. Other islands, including Rennell, Bellona, Rose, Henderson and Little Barrier, have received *RE* but not the deadlier *RN* and *RR*. These islands contain endemic species or relicts that need preserving as biological treasures. The arrival of rats on these islands could produce biological catastrophes but, contrary to common pessimistic assumptions, it is not inevitable that rats will arrive. Atkinson lists specific measures that reduce the risk. An immediate task is to secure the future of the remaining islands. □

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100 years ago

THE CONTINUITY OF THE GERM-PLASMA CONSIDERED AS THE BASIS OF A THEORY OF HEREDITY

THE present essay deals not with the entire subject of heredity, but with the fundamental question. How is it that a single cell of the body unites within itself the entire tendencies of inheritance of the whole organism? There are only two physiologically conceivable possibilities by which germ-cells endowed with such peculiar powers as we know them to possess can be produced. Either the substance of the parent germ-cell after passing through a cycle of changes required for the construction of a new individual possesses the capability of producing anew identical germ cells, or the germ-cells arise as far as their essential and characteristic substance is concerned, not at all out of the body of the individual, but direct from the parent germ-cell.

It is this latter view which Prof. Weismann holds to be correct, and maintains in the present essay, and which he terms the theory of the continuity of the Germ-plasma.

From *Nature* 33 154, 17 December, 1885

Astrophysics

Bipolar wind from young stars

from James P. Emerson

THE molecular cloud L1551 contains the best example of a collimated bipolar high-velocity molecular outflow driven by a young stellar object. Now high-resolution ^{12}CO observations have directly demonstrated the postulated windswept cavity and shell in this cloud (Snell, R.L. & Schloerb, F.P. *Astrophys. J.* **295**, 490; 1985). The complex observed morphology and kinematics can be neatly explained by the combination of two effects. First, a collimated high-velocity flow from the young stellar object IRS5 (infrared source 5) sweeps out a bipolar cavity in the molecular cloud, and compresses much of the material into a dense thin shell surrounding the cavity; and second, the cavity expands uniformly into the surrounding cloud. The biggest puzzle of all, however, still remains — what causes the collimated bipolar wind from the young star in the first place?

A key problem of astrophysics is the formation and early evolutionary stages of stars. Interpretation of the observations of young high-mass stars is particularly complicated because they are usually associated with clusters of other nearby stars, surrounding ionized hydrogen regions, ionization fronts and shocks. Therefore, many researchers hoped to get a clearer picture of star formation from studying low-mass stars forming in dark clouds, because these are relatively isolated objects and not expected to be associated with the

complexities of ionized hydrogen regions.

Far from the anticipated simplicity, there has instead been mounting evidence that energetic jets and collimated outflows are commonly associated with young stars, even those of low mass. The energy input into the surrounding clouds from these bipolar winds could significantly affect the subsequent evolution of the cloud and its star-forming history. Some of these problems are analogous to those involved in understanding jets from galaxies, although jets from young stars are non-relativistic and do not involve such great energies (see Lada, C.J. *Ann. Rev. Astron. Astrophys.* **23**, 267; 1985).

When molecular-line observers first turned their telescopes towards the candidate young star IRS5, which is in the dark cloud L1551, they were surprised to find, in addition to the expected low-velocity molecular gas associated with the dark cloud, blueshifted and redshifted high-velocity gas located in oppositely directed bipolar lobes on the sky (see figure). The south-west lobe contains blueshifted gas and the north-east lobe redshifted gas. In the approaching lobe optical nebulosity can be seen because the flow is coming out of the dark cloud, but in the receding lobe no nebulosity can be seen because of the obscuring dust.

Although many examples of bipolar molecular outflows have now been found, L1551 remains prototypical as its proximity

and orientation make it especially favourable for detailed observational study. Optical studies of the south-west lobe have identified shock-excited knots of gas (termed Herbig-Haro objects) whose proper motions are radially outwards from IRS5. Optical and high-resolution radio observations have both shown ionized jets emanating from IRS5 parallel with the much larger-scale bipolar outflow and orthogonal to a dense rotating torus of high-density material seen in CS (carbon monosulphide) observations and suggested by infrared polarization studies. Shock-excited molecular hydrogen is

found at the edge of the flow, and some of the optical nebulosity is interpreted as reflected light from IRS5.

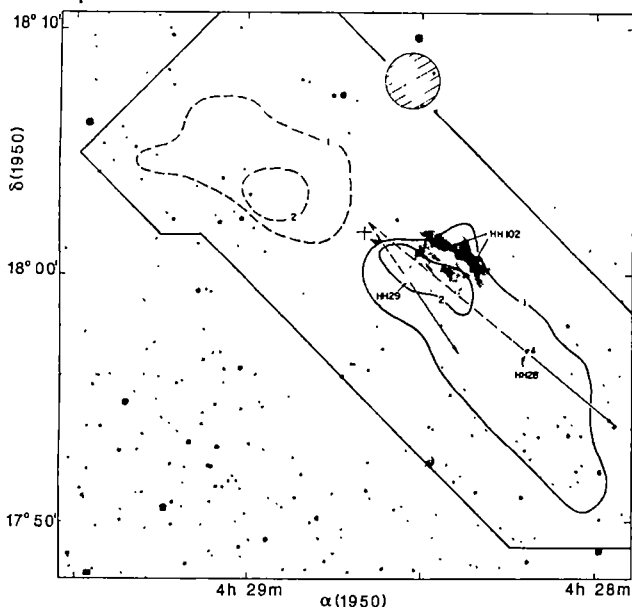
Snell and Schloerb have produced a complete ^{12}CO map of L1551 in the 2.6-mm line with the highest angular resolution ($45''$) yet used on this flow, together with lunar occultation measurements of $7''$ resolution which clearly show the cavity and shell, and demonstrate that each lobe can be considered to be divided into two velocity components. The lower velocity component is in a thin shell at the periphery of the higher velocity component and contains a substantial fraction of the 0.9 solar masses of material in the outflow. The data and the overall flow shape can be neatly and consistently interpreted in terms of the collimated bipolar wind from IRS5 driving gas radially outwards at about 12 km s^{-1} , while the bubble thus created expands uniformly at a velocity of about 4 km s^{-1} . The mass now contained in the cavity and its surrounding compressed shell is close to that expected to be swept up if the cavity volume originally contained molecular gas at the ambient density of the undisturbed central parts of the cloud.

As Snell and Schloerb point out, although the mechanical luminosity of the wind is only a small fraction of the luminosity of the embedded star, the mechanism by which the energy of IRS5 is converted into momentum in the flow is as unknown in L1551 as it is in the other examples of this phenomenon.

It is widely believed that many young stellar objects, still evolving towards the main sequence, are surrounded by remnants of their parent cloud in the form of a rotating circumstellar torus or disk. It is crucial for theories of the formation of planetary systems and stars that the disks and their interaction with the stars and their immediate environment be understood. These disks are commonly assumed to be involved in the production of jets and bipolar outflows by an interaction between the young star and the surrounding torus or disk of material. It is thought that the interaction producing the jets is mediated by some combination of local density structure, magnetic fields, rotation and accretion of material, but theoretical models have failed to provide any widely accepted explanation.

Many of the current observations make use of techniques such as interferometry and polarimetry in the infrared and millimetre spectral regions in attempts to identify clearly these disks and to define their properties and how they relate to the jets (for example, Beckwith, S. *et al. Astrophys. J.* **287**, 793; 1984 for HL Tau, which also lies in L1551), but it seems more probable that a new theoretical model is required to explain how the collimated jets from young stars are driven. □

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An earlier lower resolution ^{12}CO contour map of the L1551 outflow (from Snell *et al. Astrophys. J.* **239**, L17; 1980) in the broad-velocity components, superimposed on an optical photograph of the L1551 region. Dashed contours, redshifted molecular gas; solid contours, blueshifted gas. Also shown are the direction of the proper motions of the two compact Herbig-Haro objects, HH28 and HH29; tracing their motion backwards suggests a common origin at IRS5 (cross). α , Right ascension; δ , declination.

Drosophila development

Homing in on homoeo boxes

from Geoffrey North

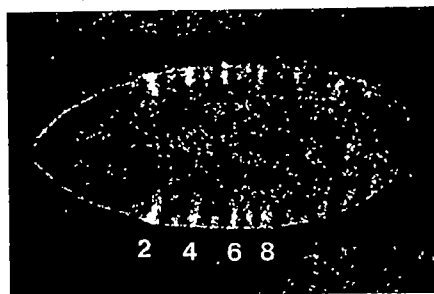
SINCE its discovery^{1,2} as a short repeated sequence within the homoeotic gene complexes of the fruitfly *Drosophila melanogaster*, research on the 'homoeo box' has taken three more or less independent directions. One has been the pursuit of evidence that the presence of homoeo boxes in the genomes of other species really does signify that they have equivalents of the fruitfly's homoeotic genes. The state of this particular art was discussed recently in these columns³. A second has been cataloguing of all *Drosophila* genes that have homoeo boxes, the newest member of the club being *caudal*⁴, which is the first example that is 'maternally expressed' and so presumably concerned with the very earliest events of development. And the third has been the attempt to ascribe a function to the homoeo box. This has now borne fruit, with the evidence reported by C. Desplan, J. Theis and P.H. O'Farrell on page 630 of this issue⁵ that the protein domain encoded by the homoeo box of the *engrailed* gene of *Drosophila* has sequence-specific DNA-binding activity.

But first some recent history. Shortly after their discovery^{1,2} as repetitive sequences of both the bithorax and Antennapedia gene complexes of *Drosophila*, homoeo boxes were found to encode roughly 60 highly-conserved amino acids, which would form a domain within the protein products of each of the genes that constitute these complexes. Although most of these genes are strictly homoeotic in that their mutations cause clean transformations of one body-part into another, one of the first homoeo boxes to be sequenced belongs to a rather different kind of gene. This is *fushi tarazu*, the odd-man out in the Antennapedia complex, which falls into the 'pair-rule' class of segmentation genes: in *fushi tarazu* mutant embryos, equivalent regions of every other segment are deleted.

The diversity of genes that contain a homoeo box was increased still further with *engrailed*^{6,7} — the first to be found outside the bithorax and Antennapedia complexes. *Engrailed* has some properties in common with the classical homoeotic genes, but others that are more reminiscent of segmentation genes such as *fushi tarazu*. Thus it seems to specify the posterior compartment of every segment of the developing fruitfly, for if clones of mutant cells lacking wild-type *engrailed* activity are created in a posterior compartment they develop as though they were in the equivalent region of the anterior compartment of the same segment. But in *engrailed* mutant embryos, unlike classical homoeotic mutants, the pattern of seg-

mentation is disrupted. In fact, the effect resembles that of a pair-rule mutation, which may relate to the observation that the developing pattern of *engrailed* expression, which in its final form is a series of stripes corresponding to the eventual pattern or morphological segmentation, goes through an intermediate stage with a clear-cut two-segment periodicity^{8,9} (see figure).

Thus, although all of the *Drosophila* homoeo-box-containing genes, including *caudal*, seem to have interesting roles in



Expression of *engrailed* with a two-segment periodicity (from ref. 8).

development, at the macroscopic level they seem to have a variety of types of function. But the implication of their shared homoeo-box domain is that at the molecular level the products of these genes work in basically similar ways. A clue as to what this common activity might be has come from the homology of the *Drosophila* homoeo boxes to regions of the yeast *MAT a* and *MAT a2* genes^{10,11}, which are known to regulate sets of genes that determine the mating type of haploid yeast cells. The protein product of the *MAT a2* gene has recently been shown to bind specifically to a sequence located upstream of the genes that it regulates¹².

It is against this background that Desplan *et al.* have tried to determine whether, as widely predicted, the *Drosophila* homoeo boxes also encode DNA-binding domains. Following a similar approach to that of Johnson and Herskowitz in their study of the yeast $\alpha 2$ protein, Desplan *et al.* have constructed fusion proteins in which different regions of the *engrailed* protein product are tagged on to the end of the enzyme β -galactosidase. Expressed from hybrid genes introduced into the bacterium *Escherichia coli*, high enough levels of these fusion proteins can be obtained for biochemical studies.

Compared with Johnson and Herskowitz, however, Desplan *et al.* were at a disadvantage, for they had no prior indication of the sequences to which the *engrailed* protein might bind. So at first they simply tested whether their fusion proteins would bind non-specifically to frag-

ments of the bacteriophage lambda genome. Indeed, they found that those fusion proteins that contained the *engrailed* homoeo-box domain did have DNA-binding activity. But was this simply due to a non-specific interaction between acidic DNA fragments and the basic protein molecules? It seems not, for under more stringent conditions it was revealed that some of the lambda fragments are bound much more avidly than others. This is a clear sign that the DNA-binding activity of the fusion proteins is of the legitimate, sequence-specific kind. Presumably it is just by chance that some lambda fragments fulfill some of the sequence requirements for binding the *engrailed* protein, but it could well be that the fusion proteins are rather less choosy *in vitro* than *in vivo* (where their specificity might well be increased by interactions with other proteins).

Finding the functionally significant sequences of the *Drosophila* genomes to which the *engrailed* protein is presumed to bind is a more difficult problem, which Desplan *et al.* have approached by following the increasing genetic evidence that genes such as *engrailed* are involved in mutual regulatory interactions. They have therefore looked for and found binding sites for the *engrailed* protein upstream of the *engrailed* gene itself — but, as the authors themselves stress, this highly intriguing result must be interpreted with caution, for the affinity of the protein for these sites is not significantly different from that for its favourite fragments of the lambda genome. The binding sites near the *engrailed* gene are conserved between two different *Drosophila* species, however, which implies some functional significance. If it turns out that they are not true binding sites for the *engrailed* protein perhaps they are binding sites for some other protein that contains the homoeo-box domain. An example would be the product of the *fushi tarazu* gene, which recent genetic evidence suggests is involved in establishing the pattern of *engrailed* expression during the early development of *Drosophila* embryos (Phil Ingham and Ken Howard, personal communication).

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Quasiperiodic order in dissipative systems

SIR—The concept of quasicrystalline order¹ in relation to experimental observations²⁻⁴ in Al_3Mn suggests that quasiperiodic structures are also likely to appear in driven systems undergoing patterning instabilities. (Classical examples are given by convective instabilities in fluids⁵ or Turing instabilities in chemical systems, for example, ref. 5). Despite the complexity of the dynamics, such symmetry-breaking instabilities may be described near threshold by a kinetic equation for the unstable or critical modes which play the role of an order parameter. They are related for example to the velocity field in hydrodynamical systems or to the concentration of active species in chemical systems^{6,7}. The stabilities of the patterns may be ordered in decreasing values of the associated Lyapunov functional which plays the role of the Ginzburg-Landau-Brazovskii potential in equilibrium situations.

Near threshold, structures built on wavevectors of critical wavelength may be stable and when the Lyapunov functional is limited to its fourth order invariant a situation very similar to the Landau treatment of crystallization is recovered⁸. Among the possible patterns one finds, for example, layered structures corresponding to modulations in one direction only, bimodal patterns and structures with triangular or cubic symmetry corresponding to combinations of critical modes with wavevectors forming equilateral triangles⁷.

However, we have to keep in mind that the kinetic equation for the order parameter-like variables is obtained via the adiabatic elimination of stable noncritical modes which are driven by the critical ones. Hence their contribution may become important for increasing values of the bifurcation parameter. As a result, the intensity of the coupling between three modes is renormalized and, furthermore, higher order nonlinearities are generated by the driven modes as in the multicomponent Landau theory⁴. The first nonlinear coupling induced by this effect in the kinetic equation involves four critical modes corresponding to a fifth order invariant in the Lyapunov functional³. This term favours the formation of structures built on wavevectors forming an equilateral pentagon which in two-dimensional systems has the symmetry of a Penrose lattice.

The emergence of high order nonlinearities in the dynamics of the critical modes favouring quasiperiodic patterns is a general feature of this description of systems undergoing symmetry-breaking instabilities^{6,7}. Hence, according to the intensities of the nonlinear couplings which are system dependent, quasiperiodic structures could become the stablest ones

in some range of the parameters. Having a dominant growth rate versus layered structures they should also appear after sudden increases of the control parameter.

In various convective instabilities, the increase of the constraint beyond threshold leads to the nucleation of defects and ultimately to the destruction of the translational order⁹. It was suggested that this effect could be related to a dislocation induced melting similar to what happens in two-dimensional solids^{9,10}. From the present analysis, however, one may also expect the appearance of quasiperiodic structures well beyond threshold. It would thus be interesting to perform diffraction experiments for such systems after slow and sudden increases of the bifurcation parameter in the region where translational order is lacking to test whether the disorganization is related to a dislocation-induced melting or to the transition from periodic to quasiperiodic structures.

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The cosmic connection of catastrophism?

SIR—The remarks by Weissman¹ on the review by Maddox² require some additional comment. This relates to a possible extraterrestrial cause of terrestrial catastrophism and, in particular, to the origin of the suggestion that this might be associated with the Sun's motion in the Galaxy.

As previously pointed out in these pages³ pulsations are not a new idea and were suggested more than half-a-century ago⁴. Later, in 1947, Umbgrove⁵ reasoned that the Earth had a periodicity of about 250 Myr and drew attention to the similarity between this and the galactic day. Although Umbgrove was probably the first to make such a suggestion, the validity of much of his evidence can certainly now be questioned⁶.

Probably the first experimental measurements relevant to this topic were carried out in the early 1960s⁷ and the re-

sults were published in this journal in 1971⁸. These measurements indicated that the times of emplacement (in Myr) of mantle-derived carbonatites (for at least the last 1.7 Gyr) could be approximated very closely by the relationship $T_n = 233n - 102$, with n integral. The similarity of this period with that for galactic rotation was then noted. The significance of the data set used in deriving this conclusion has since been tested⁹. From other evidence Innanen *et al.*¹⁰ have subsequently proposed a galactic model from which they derive an identical numerical value of 233 Myr for the length of the galactic year.

In 1971, plate tectonics was in its infancy and episodicity and global synchronicity were rather unfashionable concepts in geology. Over the years, this data set has therefore been continually expanded and improved¹¹ and incorporated into a hypothesis of Earth behaviour¹², compatible with plate tectonics, which nonetheless preserves this fundamental periodicity. This hypothesis is testable by carrying out more accurate age measurements on carbonatites.

One striking feature of the time distribution is the near coincidence of times T_1 , T_2 and T_3 with the boundaries of geological periods in Phanerozoic time¹³. It is therefore tempting to extrapolate backwards ($n \geq 4$) for a Precambrian time-scale. Another corollary of this hypothesis is that, as a large number of carbonatites exist with ages around 65 Myr (see, for example, refs 12, 14), an atmospheric spike of carbonatite-derived CO_2 may have influenced events near the Cretaceous-Tertiary¹⁵ boundary. This temporal coincidence can again be tested by accurate geochronological measurements. Whether, in addition, widespread carbonatite emplacement can explain the well-documented geochemical anomalies associated with this boundary is currently being examined by analysing rocks from (dated) carbonatite complexes which were emplaced across the boundary.

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How did we get to here from there?

Richard Wilson

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Radiant Science, Dark Politics: A Memoir of the Nuclear Age. By Martin D. Kamen.

University of California Press: 1985. Pp.348. \$19.95, £16.95.

ON August 6, 1945, I was at a camp at Titchfield, Hampshire, England, when I heard the news that an atomic bomb had been dropped on Japan. A year later I began my studies in physics for the DPhil. at Oxford. There, in the Clarendon Laboratory, I met some of those who participated in the atomic bomb programme, among them "Jim" Tuck, who had gone to Los Alamos as an explosives expert and returned to build a betatron, and Professor Franz E. Simon, who with others had worked on gaseous diffusion in the rooms below the undergraduate optics laboratory.

In that winter of 1946–1947, the severe weather prevented transport of coal from the mines to power stations for nearly six weeks; stocks were low and Britain had its first electricity rationing. As physicists we realized that this short-term problem was merely an indicator of a future long-term one, and at tea times discussed the solutions to the "energy crisis". On the one hand, we could burn coal more efficiently, and with less environmental disadvantages; gasify it underground and use the gas in a thermodynamically efficient fuel cell. And then there was nuclear power.

Since 1939 it had been clear that nuclear fission might be used either for power or for bombs. The Second World War had given work on bombs priority, but after 1945 scientists returned to their first love, nuclear electric power. The problems of proliferation of weapons among countries were clear. We concluded that within 30 years (by 1976) 30 countries could and would have bombs. But we were pessimistic: most countries that could do so have declined to make them. In another way, we were too optimistic. It never occurred to us that two countries, the United States and the Soviet Union, would possess tens of thousands each.

We were over-optimistic, too, about the speed of nuclear power development; instead of the five years expected, it took ten years to produce the first power plant (in Britain in 1956) and 25 additional years for much market penetration. We were at the same time pessimistic about the need for power. We had not anticipated that oil from the Arabian (at that time Persian) Gulf would be available at such an incredibly low cost.

We were very aware of the environmental advantages of nuclear power. Many of us had lived in London in the days of the bad fogs, and it must be remembered that the fog of early December 1952, which caused 4,000 "excess deaths", was far from the worst; it was merely the first about which there are good health statistics. At the same time we were all well aware that nuclear power is only conditionally safe — conditional on the technology being designed and operated well.

What went wrong with our projections? Why, after a promising start 30 years ago, is the nuclear power industry in such trouble in the United States? Why is the number of bombs increasing, apparently without limit? In other words, how did we get to here from there?

With all the many books about nuclear electric power that have been written, I have found none that discusses this all-important question. To illustrate this surprising omission of the world's scribblers, I discuss three of the recent books about the nucleus and man's attempts to tame it.

None of the three books gives us a clear answer. But the book by Clarfield and Wiecek, historians from the University of Missouri, strongly implies that we should abandon nuclear technology completely. This is a dangerous conclusion and unfortunately typical. Errors of fact in the book show that little reliance can be placed on the authors' justification of their case.

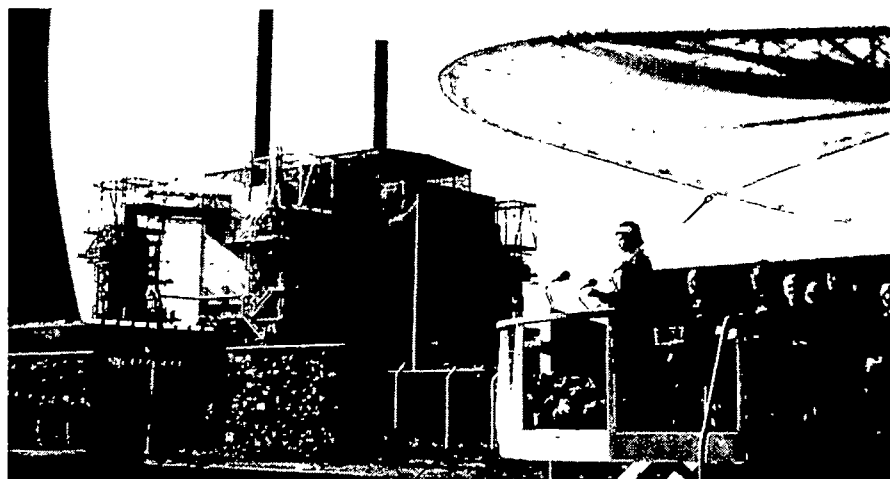
Clarfield and Wiecek consistently imply that military and civilian uses of nuclear

energy are inseparable and that we cannot stop one and not the other. This is a common view nowadays in the United States. But I regard as more tenable the proposition that military and civilian uses of nuclear power are separable and that world peace may depend on our ability to make the distinction clear. American anti-nuclear activists often damn bombs and power reactors equally; but the record shows that although activists have prevented many reactors from being built, it is doubtful whether they have reduced the military stockpiles by a single bomb. By pretending the two targets, bombs and power stations, to be equally dangerous, they have allowed themselves to be diverted from the military target and attacked the easier, more vulnerable, civilian target. Worse still, they have separated themselves from their natural allies in the scientific community and weakened the peace movement.

The layout of the book itself belies Clarfield and Wiecek's thesis. While the prologue and Chapter 1 are "mixed" chapters, about nuclear fission and its discovery, later chapters are *either* about bombs *or* about electric power reactors; not both. Only in the polemical epilogue do the two come together again.

In their discussion of nuclear safety, Clarfield and Wiecek tell their story from the outside looking in and would seem to be objective. But their technical discussions are simply wrong. Referring, as have other authors, to the Emergency Core Cooling Hearing of 1972–1973, they make the incorrect statement that "the AEC [Atomic Energy Commission] remained unmoved by these disclosures, and made no significant changes in its safety regulations". On the contrary, the AEC made enough changes to make the uncertainties disclosed in the hearing moot. The authors ignore the fact that tests of the emergency core cooling systems in 1978 showed that even the original criteria were conservative and had a margin of error.

Clarfield and Wiecek state that "Rasmussen actually had little to do with the substance" of the Reactor Safety Study of



Promising start: the Queen opens Calder Hall nuclear power station, October 17, 1956.

1975 of which he was in charge. This seems very unlikely; this year, the US Department of Energy awarded Rasmussen the Fermi Prize for his work. It is also untrue that "the Rasmussen report did not stand up well to criticism". On the contrary, the methodology is now almost universally used, and even critics rarely discuss reactor safety in any other framework. A recent study group of the American Physical Society gave it greater praise ten years later; it was found unnecessary to comment upon it specifically, but the results were used as a baseline with which to compare later calculations.

The several pages about the accident at Three Mile Island are particularly sloppy. The authors state that "the problems at TMI began when pumps forcing cooling water through the primary system tripped". It was the secondary, not the primary, pumps that tripped. The primary pumps were manually, and foolishly, shut down two and three-quarter hours later when they began to cavitate. The confusion between the primary and secondary systems is evident later in the same sentence when again they say that "temperatures began to rise in the core because the coolant was no longer flowing", clearly referring to the primary system, and then in the next sentence state that "this was a few seconds after the start of the accident". It was over two hours after the start of the accident that the primary system flow stopped and core heating really began.

More serious for a couple of historians is the uncritical mention of the worry of Dr Bridenbaugh about "the obvious use to which the government of India was putting its Tarapur reactor, the first 'peaceful' application of nuclear power in the Third World. The Indian government was reprocessing plutonium out of the spent fuel to build a bomb . . ."; this statement is almost certainly wrong. There is no evidence for it, and a lot of evidence against it. Tarapur and its fuel are under international safeguards and inspection. Both the Indian Government and the International Atomic Energy Agency say that the fuel has been stored, and the United States has a right in the agreement to take it back, unprocessed. More importantly, the reprocessed fuel would yield plutonium which has too much of the isotope ^{240}Pu , thereby making it unsuited for any but an unreliable bomb, and in India has other reactors of its own manufacture, not under safeguards, which are more suited to producing high quality, bomb grade, plutonium. Why should India break agreements and treaties, and risk being caught by an inspection, to get an inferior product?

In the second book under review, *Controlling the Atom*, I had hoped to find a clear description of the present regulations, how they led to the current situation, how France and Japan have avoided the problems, and how we might change



In defence of Martin Kamen: a cartoon in the *St Louis Star-Times*, September 4, 1948.

the situation by changing the regulations. The authors, Mazuzan and Walker, are historians at the Nuclear Regulatory Commission in Washington, and should be well equipped to do so. I do find a clear description of the present regulatory process in the United States although no comparison with its equivalents overseas. The problem arises because the book is strictly limited to the period 1946 to 1962, so that the failure of the industry in the past ten years is not even mentioned.

Nonetheless, this book makes interesting reading. The quotations make it clear that those who set up the regulatory procedures were as aware of the general safety problems as we are today. The book reminds us of the Brookhaven Study on catastrophic accidents which was presented to the Commission and released to Congress and the public on March 15, 1957, labelled WASH-740, and included the numerical estimates that some scientists gave to describe their "feeling" for the probability of accidents. Three estimates were given. Under the assumption of 100 operating reactors, and that 3,000 deaths would result from a major release outside the containment, one of them gave an individual risk of 1/50,000,000 per year of a person being killed by a reactor accident.

Eighteen years later, on February 21, 1985, I presented to the Nuclear Regulatory Commission a report prepared by a Study Group of the American Physical Society on "Radiological Consequences of Severe Nuclear Reactor Accidents". We concluded that an accident violating the containment is now much less probable than at the time of WASH-740 and although the reactors are bigger than anticipated in 1957, the consequences are likely to be smaller and 3,000 deaths would be very unlikely.

Thus it seems that the Brookhaven scientists were overly pessimistic. Although most of the American public were not interested and did not notice WASH-740 when it was released, the often-repeated

accusation that facts were withheld was, in general, untrue.

The book by Kamen, a distinguished chemist and co-discoverer of carbon-14, is a personal memoir. Kamen worked in the Radiation Laboratory at Berkeley from 1935 to 1944, and his book describes his early excitement as a scientist and his dismay at being dismissed from the Manhattan project in 1944.

It appeared later that his dismissal was not due to specific charges of disloyalty, but because he was a gregarious man with a wide circle of friends, some of whom were under suspicion. Kamen became more of a security risk than his usefulness to the project justified. Nonetheless he was denounced as "that atom spy" by the *Chicago Tribune* and *Washington Times-Herald*, but he won his libel suit against the one and the other settled out of court. His passport was withheld for seven years by Mrs Shipley, chief of the Passport Division of the US Department of State. The Department of Justice later stipulated that this was illegal. All this makes interesting reading and tells of how Americans can be misled by publicity hounds. But it also tells us another story. Bombs and power plants are only two of many uses of nuclear fission.

Kamen describes little of the scientific and technical work of the Manhattan Project in this book. It is clear that neither bombs nor electric power interest him. His scientific interests were other applications of nuclear science — first in isotopic tracers then cytochrome chemistry, biological oxidation, photosynthesis and protein structure. Radioactive isotopes in research and medical work are a third important use of nuclear fission in modern life. Kamen describes his excitement when he realized that some radioactive isotopes made for his work during the war were so radioactive that they must have been made by a nuclear reactor, rather than by a radium beryllium source. It was the medical applications of such work that earned Dr Rosalyn Yalow her Nobel Prize in medicine.

Small reactors have been built in a number of countries under the Atoms for Peace programme. It was a research reactor *not* a nuclear power reactor that was used by India to produce plutonium for a nuclear explosion. If we want to ban all nuclear technology in order to stop nuclear bombs, we would have to close down these research and isotope-producing reactors and thereby put an end to Kamen's and Yalow's research. The solution most of the world accepts is to allow both types of reactor to exist provided that there is international inspection under the Non-Proliferation Treaty. This is better than the chaos Clarfield and Wiecek would lead us to. □

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Searching for their selves

Stuart Sutherland

The Man Who Mistook his Wife for a Hat. By Oliver Sacks. Duckworth: 1985. Pp.233. £9.95. To be published in the United States in January by Summit, \$15.95.

In his latest book, the author of *Awakenings* reports many curious happenings. Perhaps the most surprising is his revelation that one can be a practising neurologist without having read the literature on visual agnosia and without knowing that retrograde amnesia is common in Kors-



Oliver Sacks: unusual neurologist.

koff's psychosis, and indeed after medial temporal lobe damage in general. Oliver Sacks asserts that he only acquired the knowledge in question after having encountered such cases himself: it is of course possible, judging from *The Man Who Mistook His Wife for a Hat*, that he had been too busy reading Wittgenstein and Nietzsche to be bothered with the literature of his profession, but one fears he may be having the reader on, a suspicion that the remainder of his book does little to allay.

Dr Sacks recounts with verve and clarity some score of neurological case-histories, all of which make excellent short stories. He describes a wide range of symptoms, ranging from Tourette's syndrome, which is marked by tics, grimaces, curses, and "an odd elfish humour . . . and outlandish kinds of play", to those of an idiot savant. His cases have only two unusual features. First, they seem to present symptoms in an exceptionally pure form and do not exhibit the messy confounding of different syndromes so common in such patients. Second, they were treated by a very unusual neurologist, Dr Sacks himself.

He is unusual in that no matter how

devastating the incapacity caused by his patient's brain damage, he always seems able to locate, usually at the first interview, an area in which the patients' "self" is still intact and which they can be encouraged to develop. This may relieve their misery — where they are miserable, since many neurological patients have no understanding of their condition and may even be unnaturally cheerful. Moreover, the development of an intact interest or skill may, according to Sacks, help to alleviate patients' symptoms. He points, for example, to a mentally retarded girl with extremely clumsy body movements, whom he helped find herself through acting: when she was on the stage all her clumsiness disappeared.

Although Sacks's grasp of brain science seems at times a little shaky (for example, Wilder Penfield did not show "beyond doubt" that epileptic transports are direct

memories of the past rather than fantasies), his message is important and compassionate. He writes, "Our tests are ridiculously inadequate. They only show us defects . . . they do not show us a being conducting itself spontaneously in its own natural way". If we are to believe him, the discovery and promotion of the spontaneous self is particularly important in neurology, but it should surely play a role in all medicine.

The book deserves to be widely read whether for its message, or as an easy introduction to neurological symptoms, or simply as a collection of moving tales. The reader should, however, bring to it a little scepticism, for outside Sacks's clinic, things do not always fall out quite so pat. □

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Different biology

Alan Bittles

Human Sexual Dimorphism. Edited by J. Ghesquiere, R.D. Martin and F. Newcombe. Taylor & Francis: 1985. Pp.375. £24, \$53.

ALTHOUGH, perhaps inevitably, there is considerable variation in the content and quality of the 18 contributions in a volume of this nature, based on a joint symposium organized by the Society for the Study of Human Biology and the European Anthropological Association, the editors have assembled an informative and very readable collection of papers on this important biological topic. From a purely semantic viewpoint, use of the term sexual dimorphism in the book is often somewhat inappropriate as the parameters can display considerable overlap between the sexes and show greater intra- than inter-group differences. The authors in general have adopted a fairly flexible, if occasionally rather arbitrary, definition for the expression of dimorphism in a species, and for this reason some of the conclusions may not necessarily be definitive.

Allometric analysis is used by a number of contributors in an attempt to unravel the circularity apparent in theories constructed to explain the origins of dimorphism. By scaling brain size to body size in simian primates it is apparent that dimorphism has evolved through reduced body size in females rather than increased male body size. Further, similar studies across a wide range of mammals have shown that brain weight is primarily associated with age at sexual maturity, rather than with lifespan as has been commonly supposed. Possible mechanisms governing embryonic sexual differentiation via H-Y antigen secreted by the Sertoli cells, and sex-differentiated behaviour

patterns are clearly presented. Given the difficulties inherent in obtaining experimental evidence from human studies, the elegant demonstration in zebra finches of a hormone-influenced dichotomous choice between male and female patterns at single neurone level, and the subsequent translation of the degree of brain masculinization into a behavioural continuum, may be of considerable significance.

While evidence from our hominid ancestors and the australopithecines relating to sexual dimorphism is both limited and literally fragmentary, the existence of dimorphic patterns in *Homo sapiens* is well established. As expected from the title, human sexual dimorphism is thoroughly assessed in a series of six papers on anthropometric, physiological and behavioural findings from a variety of extant populations in Europe, Africa and Asia. The possibility that apparent dimorphic differences may have arisen as a result of acculturation, rather than being representative of man's basal state, is illustrated in an extensive project conducted on Aboriginal hunter-gathers from Arnhem Land in northern Australia. Somewhat surprisingly, in this population the age-associated rise in blood pressure is a female, not a male, phenomenon which calls into question many of the assumptions made regarding the basic biology of our species, derived only from groups living in the developed countries.

Despite the less than perfect reproduction of the camera-ready format, overall this is an impressive publication which should find a ready audience across a wide range of biologists, behavioural scientists and clinicians. It can be recommended both as a source text and an interesting volume in its own right. □

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Feeding and flocking

C.M. Lessells

Gulls and Plovers: The Ecology and Behaviour of Mixed-Species Feeding Groups. By C.J. Barnard and D.B.A. Thompson. Croom Helm/Columbia University Press: 1985. Pp.302. £25, \$30.

THE idea that natural selection can act on an animal's behaviour, as well as its more tangible characteristics such as morphology, is well established. Behaviour is not now merely described and measured but is interpreted in terms of the selection pressures responsible for its evolution and maintenance. Barnard and Thompson follow in this tradition with their study of lapwings, golden plovers and black-headed gulls feeding on worms in agricultural pasture.

At first sight, winter feeding and flocking behaviour are really rather mundane, but this book reveals the extent of the interdependence of feeding and flocking strategies within and between the three species: foraging choices of individuals determine the size and location of feeding flocks, while the composition of flocks in turn affects feeding rates and hence the best foraging strategies. To add to this, the black-headed gulls make their living by stealing worms from the plovers, while the plovers may exploit the gulls for early

warning of the approach of predators.

Clearly Barnard and Thompson have set themselves quite a task in unravelling the complexities of this system. Their quantitative approach to the problem relies on a wealth of data, so much so that the book is rather hard going. Ultimately the success of the book must be judged by whether they are able to cut a path through the jungle of tables and graphs.

As in many other studies, the difficulties firstly of assessing costs and benefits of alternative actions, and secondly of reconciling the different units (such as calories and probability of predation) of costs and benefits, have made quantitative predictions rather thin on the ground. Precise quantitative predictions, however, can be made about the choice of food items by feeding plovers and thieving gulls. The "optimal diet model" provides a well-worked solution to both problems, but here it has been mangled almost beyond

recognition. Profitability, encounter rate, availability and prey types are all incorrectly defined; for instance equation (8.2) is akin to saying that the overall effect of driving for a while at 20 mph and a while at 30 mph is driving at 50 mph. Consequently the line of argument is weakest where it should be strongest. Similarly, innovative thinking elsewhere in the book is marred by inadequate explanation, confused terminology or misused statistics.

Despite this there is much interesting new detail in the book and some new ways of looking at the interrelationships of flocking and foraging strategies. Barnard and Thompson would have contributed more, however, if in their genuine attempt to push back the frontiers of darkness they had not extinguished the lights behind them. □

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Isolated objectives

C.J. Marshall

Molecular Cell Genetics. Edited by Michael M. Gottesman. Wiley: 1985. Pp.931. £82.30, \$79.95.

MUCH of the work on the genetics of cultured cells has made use of Chinese hamster cells, especially the CHO cell line originated by T.T. Puck, the founder of cell genetics. The importance of these cells is reflected in the emphasis of this book and it is worth noting that, as Yerganian describes, the use of Chinese hamsters is not without heroic and even tragic episodes — during the Revolution a Chinese research worker was imprisoned for his involvement in the shipment of animals to the United States.

The reasons why the CHO cell line has been used so extensively are partly historical, but perhaps more important is the comparative ease with which recessive phenotypes can be isolated in this cell line. Siminovitch, in 1976, proposed that recessive mutations could be isolated in CHO cells because some loci were hemizygous as a result of chromosome rearrangements.

Subsequent studies described by Siminovitch and others have confirmed and extended these ideas. For some autosomal loci, CHO does appear to have only one copy of the genes; however, other loci are diploid and there is increasing evidence that one locus can be inactivated or deleted by a high frequency event (10^{-3} or greater) to uncover recessive mutations. Such events may have profound implications for our understanding of alterations in gene activity that lead to neoplastic transformation.

In *Molecular Cell Genetics*, the editor lists over 100 mutant phenotypes in

Chinese hamster cells which include nutritional auxotrophic requirements, mutations in mitochondria, RNA polymerases, protein synthesis, DNA repair, lysosomes, membrane proteins, glycosylases, protein kinases and many others.

As is to be expected of a multi-authored book, the chapters in *Molecular Cell Genetics* are of variable quality. Some authors have written overviews of an area, often drawing very heavily on their own studies; others have provided both a review and useful hints for particular procedures. In most cases detailed experimental protocols are not provided, so this is not a laboratory manual but rather a source book which would enable one to find the appropriate protocol. Over half the chapters are concerned with particular mutant systems, while others describe features of Chinese hamster cells and methods for cell genetics such as cell fusion, gene transfer, and cloning and expression of cDNAs. Much of the work still appears to be at the stage of characterizing mutants rather than using them to dissect a particular system or probe the structural function of a protein.

For much of the book I felt that a great deal was expected of the next development: the isolation of the relevant genes. For some loci this has already been achieved; however, there is still much to be done before many of the mutant phenotypes can be exploited for what the editor terms "molecular cell genetics": the synthesis of somatic cell genetics with molecular biology.

For those working in this area, or thinking of entering it, this book will be a valuable source of information. They might like to bear in mind, however, that there is more to cell genetics than *Cricetulus griseus*. □

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Editors: **E. L. Reinherz**, Boston, MA; **B. F. Haynes**, Durham, NC; **L. M. Nadler**, Boston, MA;
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Leukocyte Typing II summarizes the results of the Second International Workshop on Human Leukocyte Differentiation Antigens. This effort involved all research groups active in the field of human leukocyte antigens defined by monoclonal antibodies. This extraordinarily broad-based study was sponsored by NIH, WHO, and IUIS. The results of this analysis will be the basis for recommendations regarding standardization and nomenclature on workshop results.

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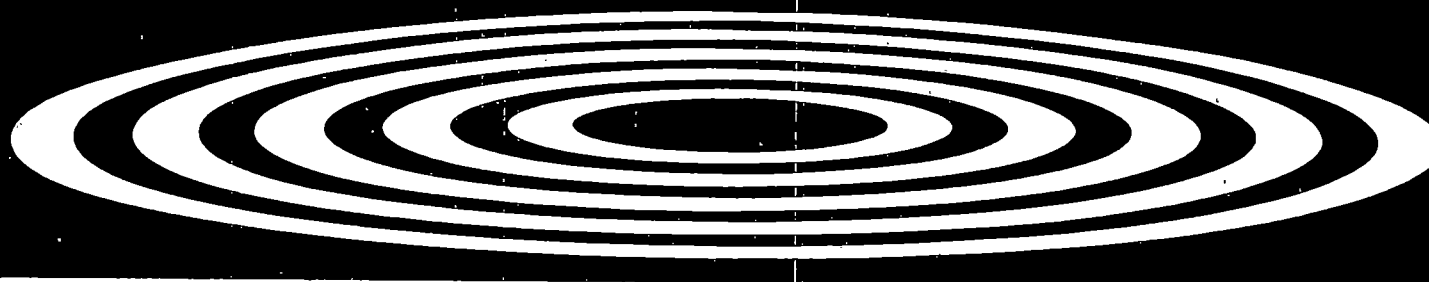
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MOLECULAR BIOLOGY BECOMES BIOTECHNOLOGY

Localizing active sites in zeolitic catalysts: neutron powder profile analysis and computer simulation of deuteropyridine bound to gallozeolite-L

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The location of a guest organic base within a model zeolitic catalyst can be quantitatively determined from an analysis of the neutron powder diffraction profile. The calculated position and orientation of the guest species within the channel agree well with the experimental values.

THE analogy between the mode of action of enzyme catalysts and zeolites is more than superficial. In each case, folds or cavities in the catalysts impose shape-selectivity that governs the 'choice' of reactant species, and the molecular complementarity of the microenvironment of the active site facilitates ensuing chemical conversion. Paradoxically, progress in characterizing the nature of active sites in enzymes (and, therefore, in engineering new proteins^{1,2}) is more advanced than that in characterizing the precise nature of the active site in zeolites. Notwithstanding their greater molecular complexity, enzymes can be made to yield good-quality single crystals which are then amenable to X-ray crystallographic analyses. Moreover, since the advent of genetic engineering, theories of enzyme catalysis in general, and views about active sites in particular, can be tested by delicate alteration of the enzyme structure. With zeolites, however, the situation is rather different. Although rapid progress has recently been made in the synthesis of new zeolitic catalysts³⁻⁵, it is very difficult to prepare them as defect-free, single crystals of a size and quality that permits X-ray analysis of the reactant-catalyst complex. Consequently, it has not yet been possible to emulate, using aluminosilicate zeolitic catalysts, work such as that done to locate fructose-6-phosphate and ATP at the active site of fructophosphokinase^{1,2}.

Alternative approaches to the elucidation of the structure of zeolitic catalysts have, therefore, been evolved⁶⁻²². These include application of such techniques as high-resolution electron microscopy⁶⁻¹¹, atom-atom calculations^{12,13}, magic-angle spinning NMR (MAS-NMR)¹⁴⁻¹⁷, and neutron¹⁸⁻²² and X-ray^{23,24} powder profile analysis. Of these techniques, the neutron profile method yields quantitative atomic structural information; it has been used to determine the location of the detachable proton in the (Brønsted)-active site in La³⁺ ion-exchanged zeolite-Y catalyst²¹, and also the siting of guest species such as Xe (which serves as a model for methane) in zeolite-rho²², and CO in zeolite-A²⁵. The precise location of sorbed benzene in zeolite-Y has also been determined by neutron powder profile analysis²⁶. Here we identify in atomic detail the siting of pyridine (which, together with other nitrogenous bases such as 4-methylquinoline²⁷, is used to block active sites) in the channels of a synthetic zeolite-L, and compare our results with a computer simulation based on atom-atom potentials.

Neutron powder diffraction analysis

The particular catalyst preparation used is a potassium salt of a gallosilicate-L, in which Ga replaces Al in the well-known structure (Fig. 1) of the anion framework²⁸. Chemical analysis gave a unit cell composition of K_{10.3}Ga_{10.3}Si_{25.7}O₇₂ for the dehy-

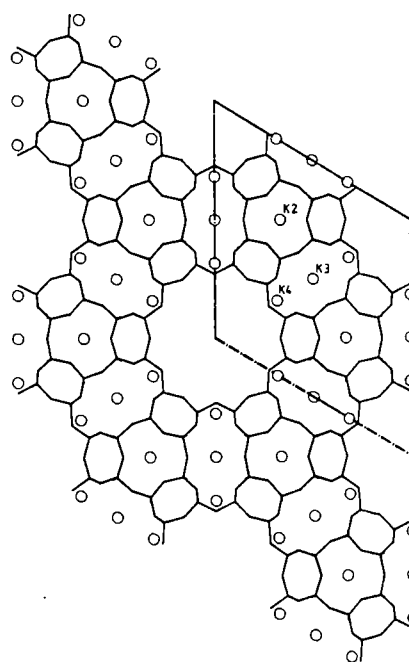


Fig. 1 A schematic representation of the structure of potassium zeolite-L, viewed down [001]. The aluminosilicate framework is indicated by lines and the cation positions as small circles. The large channel runs parallel to [001].

drated sample. Samples were characterized by X-ray diffraction and by MAS-NMR. High-quality, microcrystalline samples of the potassium salt (henceforth designated KGaL) yielded good neutron diffraction (powder) patterns, both in the dehydrated state and after incorporation into the dehydrated solid of ~1.2 molecules of perdeuteropyridine per unit cell. Data were recorded at 4 K at the Institut Laue-Langevin (ILL), Grenoble, using the high-resolution powder diffractometer, D1A.

There were some 70 discernible peaks in the 2θ -range with a neutron wavelength of 1.909 Å. Refinement of the structure, using Rietveld profile analysis^{18,19}, was done as follows. First, the starting coordinates of a previous refinement of KAIL²⁸ were assumed for the KGaL. Scattering lengths were taken from Bacon²⁹: $b_O = 0.575$, $b_N = 0.940$, $b_C = 0.663$, $b_K = 0.37$, $b_D = 0.67$, all in units of 10^{-14} m. The scattering of the tetrahedral sites was calculated from a weighted average of the scattering lengths of silicon ($b_{Si} = 0.415$) and gallium ($b_{Ga} = 0.720$) and the occupancies refined. Refinement progressed smoothly; the occupancy factors of the two distinct tetrahedral sites were

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Table 1 Atomic coordinates, isotropic thermal parameters and occupancies (number per unit cell, *N*) for KGaL (A) and KGaL+pyridine (B)

Atom	<i>x</i>	<i>y</i>	<i>z</i>	<i>B</i>	<i>n</i>
A					
T-1	0.0933(4)	0.3569(4)	0.5000	0.1(1)	12
T-2	0.1672(4)	0.5001(4)	0.2128(4)	0.1†	24
O-1	0.0000	0.2744(8)	0.5000	2.7(1)	6
O-2	0.1666(4)	0.3332(3)	0.5000	3.3(1)	6
O-3	0.2658(2)	0.5316(4)	0.2617(8)	2.1(1)	12
O-4	0.1011(4)	0.4145(4)	0.3184(8)	3.2(1)	24
O-5	0.4258(2)	0.8516	0.2790(10)	2.2(1)	12
O-6	0.1469(4)	0.4795(4)	0.0000	2.5(1)	12
K-2	0.3333	0.6667	0.5000	2*	2*
K-3	0.0000	0.5000	0.5000	2	3.0(1)
K-4	0.0000	0.3222(10)	0.0000	2	5.2(2)
B					
T-1	0.0946(6)	0.3587(8)	0.5000	0.7(1)	12
T-2	0.1658(8)	0.4987(6)	0.2125(10)	1.2(1)	24
O-1	0.0000	0.2724(10)	0.5000	2.2(1)	6
O-2	0.1654(4)	0.3308(10)	0.5000	3.1(1)	6
O-3	0.2650(4)	0.5297(8)	0.2662(14)	1.7(1)	12
O-4	0.1006(5)	0.4148(6)	0.3187(10)	3.5(1)	24
O-5	0.4267(4)	0.8535(8)	0.2770(16)	3.4(1)	12
O-6	0.1464(6)	0.4781(6)	0.0000	2.3(1)	12
K-2	0.3333	0.6667	0.5000	2.0	2*
K-3	0.0000	0.5000	0.5000	2.0	2.9(1)
K-4	0.0000	0.3188(16)	0.0000	2.0	5.3(1)
Pyridine					
D-1	0.1228(18)	0.2129(10)	0.2630	3.4(4)	3.0(2)
C-1	0.0908(18)	0.1967(10)	0.1530	3.4(4)	3.0(2)
N-1	0.1333(18)	0.2192(10)	0.0000	3.4(4)	1.5(1)
C-2	0.0045(18)	0.1534(10)	0.1580	3.4(4)	3.0(2)
C-3	-0.0454(18)	0.1284(10)	0.0000	3.4(4)	1.5(1)
D-2	-0.0212(18)	0.1404(10)	0.2700	3.4(4)	3.0(2)
D-3	-0.1042(18)	0.0992(10)	0.0000	3.4(4)	1.5(1)

Refinement parameters were as follows. A: $R_w = 8.3$, $R_p = 4.48$; $a = 18.6673(1)$, $c = 7.4956(1)$; asymmetry parameter = 0.31(1); $U = 0.58(2)$, $V = -1.03(2)$, $W = 0.69(1)$; no. of variables = 39; range refined = 10–120° 2 θ . B: $R_w = 12.0$, $R_p = 11.3$; $a = 18.6314(7)$, $c = 7.5081(4)$ Å; asymmetry parameter = 0.2(1); $U = 0.62(3)$, $V = -1.00(4)$, $W = 0.68(1)$; no. of parameters = 41; range refined = 10–110° 2 θ . Space group²⁸ P6/mmm.

* Constrained.

† Constrained to have same value as T-1.

varied independently, but they showed no evidence of Ga/Si ordering of the kind found in zeolite-A²⁰ for the Si/Al tetrahedral sites. The K⁺ ions in KGaL are located in precisely the sites that they occupy in KAlL²⁸. The final profile (R_p) and weighted profile (R_w) values were 7.5% and 8.3% respectively. Structural details are given in Tables 1A and 2.

Initial refinement of the data for the perdeuteropyridine complex was carried out using the model for KGaL, and a difference Fourier calculation showed that there was appreciable scattering from within the main channels. Recognizing (from infrared evidence³⁰) that the nitrogen of the pyridine is sited close to a K⁺ ion, and that the pyridine probably retains its planarity and other structural features of the free molecule, several model structures were generated—first with the plane of the pyridine initially parallel to (010), then parallel to (001) and to several other angles of tilt with the molecule parallel to (001). A model with the molecule tilted 24° with respect to (010) refined to high occupancy and yielded an encouraging improvement in R_w . Continuing in this manner, and allowing the K-4 site of potassium and framework sites to relax, while retaining N-1, C-3 and D-3 on the (001) mirror plane, a refinement (with an R_p and R_w of 11.3% and 12.00% respectively) was obtained indicating 1.5 molecules of pyridine per unit cell (Fig. 2). At all stages, the pyridine was constrained to the structure of the free molecule³¹. Attempts to refine the structure with the molecule

Table 2 Bond lengths and angles

	KGaL	KGaL+pyridine.	
T-1—O-1	1.650(8)	1.691(12)	
T-1—O-2	1.635(15)	1.646(21)	
T-1—O-4(2)	1.695(8)	1.686(13)	
Mean T-1—O	1.669	1.676	
T-2—O-3	1.669(8)	1.687(16)	
T-2—O-4	1.652(8)	1.630(12)	
T-2—O-5	1.664(12)	1.670(12)	
T-2—O-6	1.640(3)	1.639(8)	
Mean T-2—O	1.656	1.656	
T-1—O-1—T-1	132.1	129.0	
T-1—O-2—T-1	152.9	148.2	
T-2—O-3—T-2	135.8	135.3	
T-1—O-4—T-1	141.6	140.9	
T-2—O-5—T-2	139.1	139.7	
T-2—O-6—T-2	153.1	153.7	
Cation coordination to framework oxygens			
K-2—O-3(×6)	2.821(6)	2.821(12)	
K-3—O-5(×8)	2.915(7)	2.897(13)	
K-4—O-4(×4)	2.996(8)	3.014(7)	
K-4—O-6(×2)	2.844(8)	2.855(8)	
Interatomic distances from the pyridine atoms to the zeolite			
N-1—K-4	3.00(3)	D-2—O-1	2.87(2)
C-1—K-4	3.63(3)	C-1—O-1	3.74(3)
C-2—K-4	3.34(2)	C-2—O-1	3.42(2)

No estimated standard deviations were calculated for the framework angles; they are in 1° for each angle.

* In the literature there is talk of another site for the potassium ion, labelled K-1, for which we find no evidence in this study.

placed at other positions, for example as a π complex with K-4, gave a substantially higher R factor ($R_p = 15.5\%$). The final observed and calculated profiles for both KGaL and the zeolite-pyridine complex are shown in Fig. 2. The structural details are given in Tables 1B and 2.

Figure 3A shows the location of a single pyridine molecule in the main channel of zeolite-L. The nitrogen atom is coordinated to potassium, K-4, but the molecule also lies close to the channel wall, thus optimizing its interaction with the zeolite framework. On general chemical principles, coordination of alkali metals by ammine ligands is rare; but clearly the open, 6-fold coordination of K-4 in this dehydrated system offers an attractive location for the basic pyridine molecule. The observed K—N distance, 3.00 (± 0.03) Å, is consistent with metal—nitrogen distances found in amines of NaCl (Na—N ~ 2.6 Å³²).

A comparison of our two refinements shows that the framework structure of the zeolite appears to be essentially unperturbed by the presence of the pyridine molecule, but this conclusion may be misleading because, on average, only 1.5 of the six potassium sites K-4 in each cavity are coordinated. Only a fraction of the framework atoms around the channel will therefore respond to the close proximity of pyridine. However, the position of K-4 does appear to have altered slightly on complexation with the pyridine.

Computer simulation

We have also explored the possibility of calculating the minimum energy position for pyridine in zeolite-L, using atom-atom potentials according to the method of Ramdas *et al.*³³. Kiselev, whose parameterization was used in the present work, has already shown^{34,35} that such calculations yield enthalpies of adsorption that are in good agreement with experimental data. We used the program 'Chemgraf'³⁶. The calculation models the short-range (van der Waals) interactions and the electrostatic terms (dipolar and higher order, in this instance) between the pyridine and the zeolite, but it does not include any covalent contribution to the K—N bond energy—this, of course, is expected to be small. A single molecule was placed as in Fig. 3A and the interaction energy was calculated as a function of its

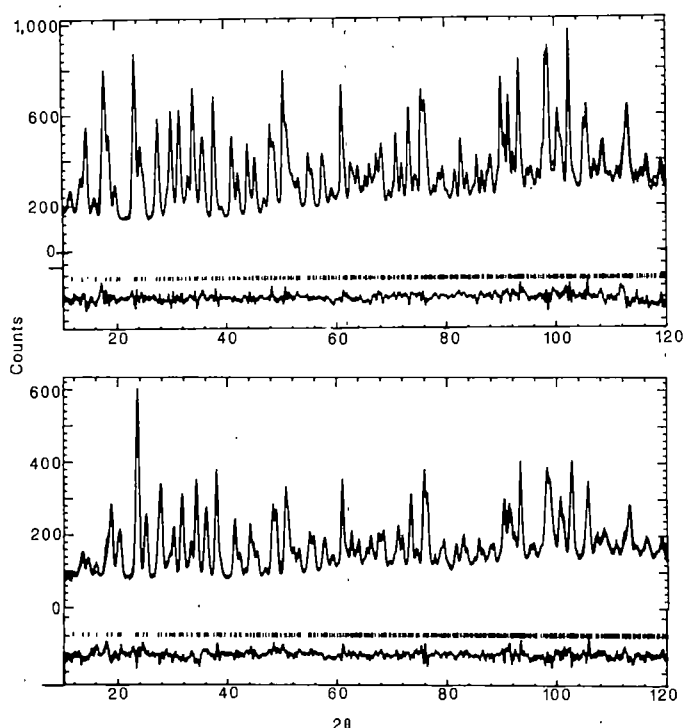


Fig. 2 Neutron powder diffraction profiles of *A*, KGaL and *B*, the KGaL-perdeuteropyridine complex. The upper curves show the observed and calculated profiles—these are generally coincident within the resolution of the plotter. The lower curve shows the difference profile. The vertical lines mark the peaks used in refinement (up to 2θ of 120°).

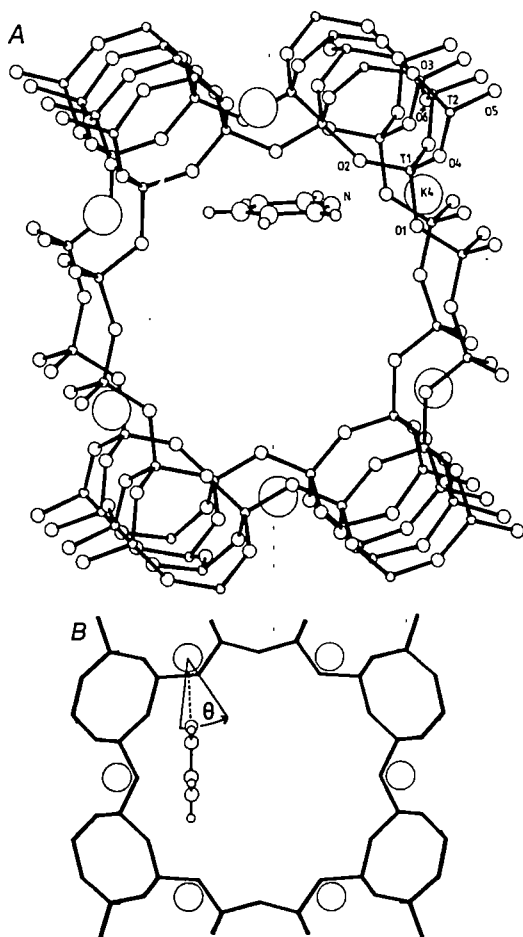


Fig. 3 *A*, The location of a single pyridine molecule in the large channel of zeolite-L, viewed approximately down [001]. *B*, A view of the pyridine molecule down [001], showing the angle θ which was varied for the calculations shown in Fig. 4A.

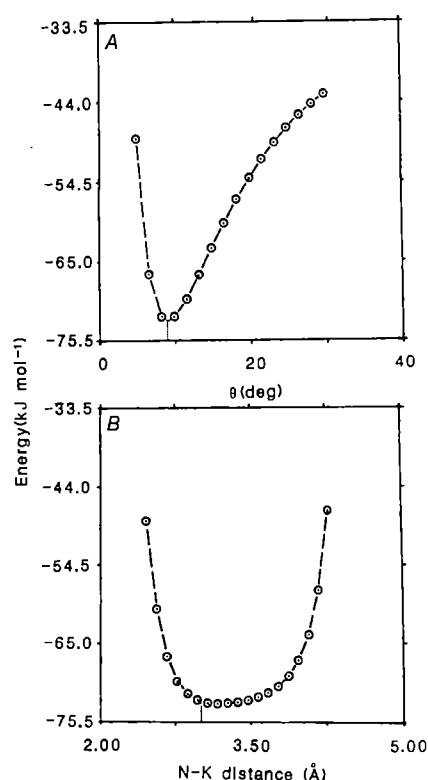


Fig. 4 The interaction energy between pyridine and the zeolite as a function of (*A*) the angle θ and (*B*) the K-4—N distance at $\theta = 8.8^\circ$; the observed positions are indicated by dashed lines.

angular position θ (Figure 3*B*) and the K-4—N distance at $\theta = 8.8^\circ$; the calculations yield an energy minimum at this θ value (Fig. 4*A*) that is in excellent agreement with the observed value of 8.6° , and a K-4—N distance of 3.13 \AA (Fig. 4*B*). The predicted position of the molecule is within 0.2 \AA of the observed location.

Discussion

It is worthwhile emphasizing that exclusive occupancy of the minimum enthalpy position of the molecule is likely to be found only at very low temperatures, as used in the present work, where entropy considerations are minimized. This factor underlines one of the great advantages of powder neutron diffraction methods—the ease with which data can be collected over a wide temperature range. The concordance between our experimental and theoretical results lends credence to the efficacy of both methods, and more ambitious aims may now be within reach. These include the simulation of catalytic reactions in zeolites, by using a combination of molecular mechanics and molecular orbital calculations, the experimental location of catalytic intermediates, and—again drawing on the enzyme analogy—the study of sorbate motion in zeolites by molecular dynamics.

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New constraints on transient lower mantle rheology and internal mantle buoyancy from glacial rebound data

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A model of mantle rheology which has an explicit transient component of its relaxation spectrum is shown to reconcile two apparently conflicting inferences of lower mantle viscosity. If recent analyses of isostatic geoid anomalies are correct in requiring a large increase of viscosity with depth, then the weak viscosity stratification demanded by postglacial rebound data implies the importance of such rheological behaviour, at least in the Earth's lower mantle.

OVER the past decade a large number of diverse geophysical observations have been shown to be intimately connected with the process of glacial isostatic adjustment. These include relative sea-level histories in the age range 0-18 kyr before present, free-air gravity anomalies over existing centres of postglacial rebound, and certain anomalies in the Earth's rotation including the non-tidal component of acceleration of the axial rotation rate which has recently been confirmed (through J_2) by laser ranging to the LAGEOS satellite^{1,2}, and the wander of the rotation pole with respect to the surface geography which is revealed in the International Latitude Service path to be occurring at a rate near (0.95 ± 0.15) degrees per 10^6 yr towards eastern Canada.

The demonstration of the intimate connection which exists between these phenomena has been provided through systematic application of the linear viscoelastic field theory of glacial isostasy³. Detailed recent accounts of the application of this theory to the four types of data mentioned above have appeared in several recent articles⁴⁻⁷. All of the analyses described in these papers were predicated on the assumption that the mantle could be described as a linear Maxwell solid insofar as its viscoelastic behaviour was concerned. The main result of these analyses has been the demonstration that in the context of this rheological parameterization, the viscosity of the mantle had to be rather uniform, with a lower mantle value of $2-3 \times 10^{21}$ Pa s and an upper mantle value close to 10^{21} Pa s. Furthermore, relative sea-level data from beyond the margin of the Laurentian ice sheet can be invoked⁸ to constrain the thickness of the continental lithosphere to a value near 200 km, and several articles^{5,7,9} have established that in order to explain observed free air gravity anomalies with the same model as is required to fit sea-level observations, the internal density stratification of the mantle must be allowed to deliver a buoyant restoring force when the individual horizons within it are deflected from their equilibrium levels in the isostatic adjustment process. This latter inference is obviously of interest from the point of view of theories of the mantle convection process.

Transient rheology and glacial isostasy

Weertman¹⁰ first drew attention to the possibility of a conflict between the uniform viscosity mantle implied by glacial rebound

data and the highly non-uniform viscosity mantle expected on the basis of the microphysical theory of solid-state creep in the Earth. He suggested that the resolution of this impasse may be simply to allow that the lower mantle viscosity to which the rebound process was sensitive is a transient value rather than the steady-state value which is assumed in the Maxwell analogue. Until recently it has remained equally plausible that the fundamental assumptions underlying the microphysical model might be the cause of the discrepancy between the expected value of the lower mantle viscosity and that required by glacial rebound observations.

Recent analyses of isostatic geoid anomalies by Hager¹¹ and Richards and Hager¹² appear to point to the validity of Weertman's interpretation, however, because these anomalies, presumably forced by the density heterogeneity associated with mantle convection, are interrogating the viscosity spectrum on a characteristic timescale of the order of 10^8 yrs. Richards and Hager found that they could fit the lowest degree components of the geoid height spectrum ($l=2$ and $l=3$) on the basis of the internal heterogeneity of density inferred from seismic tomographic analyses^{13,14} only if the lower mantle were about a factor of 10 more viscous than the upper mantle. Given the simplicity of the model used to make this inference, the increase in the viscosity contrast preferred by the longest wavelength geoid height data above that required by glacial rebound observations may or may not be significant.

In order to obtain a similar reconciliation of geoid height anomalies in the spectral range $4 \leq l \leq 9$, however, Richards and Hager were obliged to add the lateral heterogeneity of density associated with downgoing slabs in oceanic subduction zones to that supplied by objective seismic tomography, since the latter technique is as yet insensitive to these smaller wavelength features when applied on a global basis. To best fit the pattern and amplitude of the slab scale geoid height features then required a very substantial further increase in the contrast of viscosity between upper and lower mantle. Recently stated preferences for the contrast have reached a factor of 10^2 or more. In this article I wish to point out an important implication of this result, if it is in fact correct, and to demonstrate through explicit analysis the way in which it would have to be reconciled with previous work on the problem of glacial

isostatic adjustment if it should be verified by further investigation.

There have in fact been several recent re-analyses of post-glacial rebound phenomena, inspired in part by this result, using a rheological parameterization which explicitly includes transient relaxation of the lower mantle as was suggested originally by Weertman¹⁰ to be necessary on other grounds. If such additional rheological complexity were manifest it would clearly explain in a rather simple way why the viscosity of the lower mantle 'seen' by short timescale glacial rebound phenomena might be significantly lower than the viscosity controlling the longer timescale mantle convection process. The Burgher's body model of Peltier *et al.*¹⁵ has recently been used by several authors¹⁶⁻¹⁸ to represent the hypothesized lower mantle transient. The main conclusion derived from this work is that such models might provide equally acceptable fits to the data as those previously delivered by the Maxwell models in which the relaxation spectrum contains no transient component. In this article I demonstrate that, when all of the signatures of the isostatic adjustment process are invoked simultaneously, it is possible to restrict very considerably the class of such transient models allowed by the data. In particular it will be demonstrated that acceptable models in the allowed class are those which essentially behave as Maxwell models and that strong internal buoyancy is still required within the mantle if they are to reconcile simultaneously both relative sea-level and free-air gravity observations.

Burgher's body rheology

The frequency-dependent shear modulus of a three-dimensional Burgher's body rheology has the form¹⁵:

$$\mu(s) = \frac{\mu_1 s}{(s + \mu_1/\nu_1)} \left[\frac{(s + \mu_2/\nu_2)(s + \mu_1/\nu_1)}{(s + \mu_2/\nu_2)(s + \mu_1/\nu_1) + \mu_1 s/\nu_2} \right] \quad (1)$$

in which s is the Laplace transform variable, μ_1 is the elastic shear modulus, ν_1 the steady-state viscosity, and μ_2 and ν_2 are the shear modulus and viscosity of the Kelvin-Voigt element of the Burgher's body which determine the strength and timescale of the transient relaxation which it supports. Note from equation (1) that in the limit $\nu_2 \rightarrow \infty$ the Burgher's body degenerates to a Maxwell solid with $\mu(s) = \mu_1 s / (s + \mu_1/\nu_1)$ which is the same analogue as has been conventionally used in postglacial rebound analyses³. More important for our present purposes is the limit $\mu_2 \rightarrow 0$ in which case the analogue again degenerates to a Maxwell solid but in this limit $\mu(s) = \mu_1 s / (s + \mu_1/\nu_{\text{eff}})$ where the effective viscosity $\nu_{\text{eff}} = \nu_1 \nu_2 / (\nu_1 + \nu_2)$. This is important because it means that in the limit of large defect ($\mu_2 \ll \mu_1$) the relaxation will proceed as if it were a steady-state relaxation, and to the extent that $\nu_1 \gg \nu_2$ the effective viscosity governing the rate at which relaxation proceeds will be the transient viscosity since $\nu_{\text{eff}} \approx \nu_2$ in this limit. This article addresses the question of whether transient rheologies which have only modest elastic defect, for which the condition $\mu_2 \ll \mu_1$ does not apply, are viable models for the explanation of isostatic adjustment data. The results presented below demonstrate that they are not and therefore that previous inferences of effective lower mantle viscosity based upon application of the Maxwell analogue may simply be interpreted as representing a transient element of the viscosity spectrum if this should prove necessary. Although transient models which are allowed by the data are able to fit free-air gravity observations with somewhat less internal mantle buoyancy than was required with the Maxwell analogue, non-negligible buoyancy is still required.

For purposes of the present discussion we will assume that only the lower mantle has a transient component in its relaxation spectrum and therefore will take $\nu_2 = \infty$ in the upper mantle over the depth range 0-670 km. This is not meant to imply that transient relaxation of the upper mantle can be excluded on any *a priori* grounds but only that we intend to illustrate the influence of transient rheology in terms of a model in which its effect is confined to the lower mantle. We will also assume that the steady-state viscosity of the upper mantle is 10^{21} Pa s and

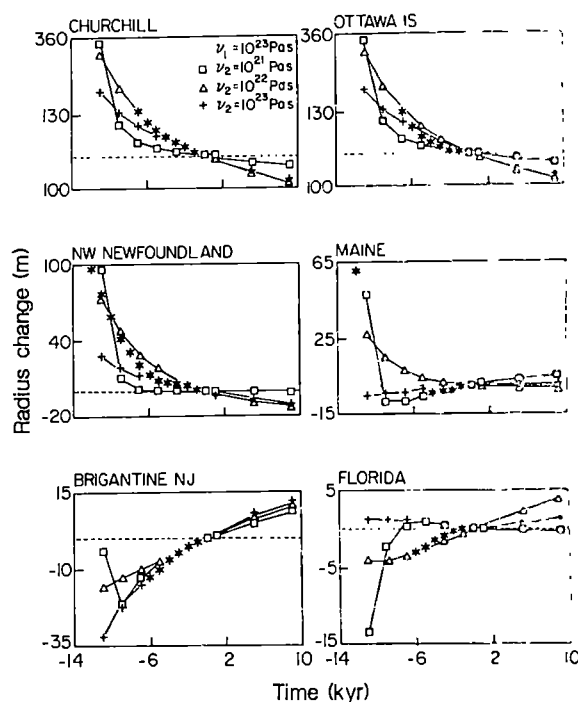


Fig. 1 Comparison of observed (*) and predicted relative sea-level data at six North American sites for the Burgher's body rheology with $\mu_2/\mu_1 = 0.1$. The lithospheric thickness of the Burgher's body is fixed at 120.7 km, $\nu_1 = 10^{21}$ Pa s and $\nu_2 = \infty$ in the upper mantle, and $\nu_1 = 10^{23}$ Pa s in the lower mantle. The different relative sea-level predictions on each plate are for models with lower-mantle ν_2 values of: 10^{21} Pa s (\square), 10^{22} Pa s (\triangle), 10^{23} Pa s (+).

the lithospheric thickness 120.7 km. Between the seismic discontinuity at 670 km depth and the core-mantle boundary, we will, for purposes of illustration, accept the results of Richards and Hager¹² as fixing the steady-state creep resistance of the lower mantle to the value of 10^{23} Pa s, that is, two orders of magnitude higher than the upper mantle value. We will then invoke the four previously discussed signatures of the isostatic adjustment process to constrain the remaining parameters μ_2 and ν_2 which are required to complete the description of the rheology of the lower mantle. The elastic structure upon which the above viscous layering is superimposed will be assumed identical to the model with two internal mantle density discontinuities which I have used previously⁸ which as a 3.8% increase of density at 420 km depth and a 6.2% increase at 670 km depth. In the analysis of free-air gravity anomalies we will compare the predictions of this model to one which has no internal mantle density discontinuities and therefore no source of internal mantle buoyancy.

Transient relaxation and postglacial rebound

Figure 1 shows observed and predicted relative sea-level variations at six North American sites for the Burgher's body rheology with $\mu_2/\mu_1 = 0.1$ and for three different choices of the lower mantle ν_2 ($=10^{21}$ Pa s, 10^{22} Pa s and 10^{23} Pa s) with other parameters of the model fixed to the values stated above. All calculations are based upon the circular disk load approximation to the Laurentian ice sheet with parameters listed in ref. 8 and include the influence of seven prior 10^5 -yr cycles of glaciation and deglaciation as may be inferred to have occurred on the basis of $\delta^{18}\text{O}$ data from deep-sea sedimentary cores. Inspection of these comparisons demonstrates that at sites within the ice sheet margin like Churchill, Ottawa Islands, and north-west Newfoundland, the data prefer 10^{21} Pa s $< \nu_2 < 10^{22}$ Pa s with the lowest value strongly rejected because it leads to overly rapid initial relaxation and thus severely underestimates present day emergence rates. More detailed analysis demonstrates that the preferred ν_2 at Churchill is 5×10^{21} Pa s, whereas that at both the Ottawa Islands and north-west Newfoundland sites is closer to $2-3 \times 10^{21}$ Pa s. At the Maine site which is on the ancient

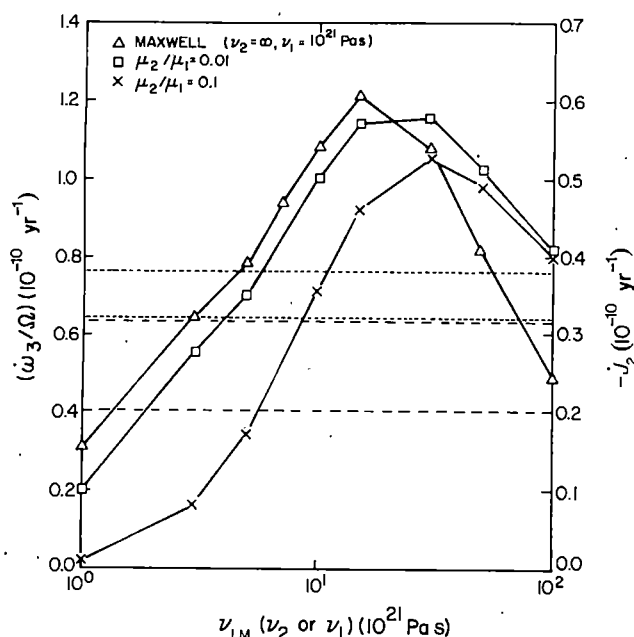


Fig. 2 Predicted J_2 and the associated non-tidal acceleration of rotation as a function of lower-mantle viscosity for both the Burgher's body rheologies with $\mu_2/\mu_1 = (0.1, 0.01)$ and for the Maxwell analogue to which the Burgher's body degenerates in the limit $\mu_2 \rightarrow 0$. The upper and lower horizontal dashed lines on this plate correspond respectively to the observed values based upon the analyses of Lageos data by Yoder *et al.*¹ and Rubincam².

margin of the Laurentian ice sheet, high values of ν_2 eliminate the non-monotonic signature from the predictions and therefore are excluded by the data. As was found to be the case with the Maxwell analogue, even a value of 5×10^{21} Pa s for lower mantle ν_2 is unacceptable from the point of view of such data. At Brigantine further south (see ref. 8 for a location map) the data are not as sensitive to lower-mantle viscosity. As shown previously⁸, however, the observations at such sites which are near the crest of the glacial forebulge are extremely sensitive to lithospheric thickness. The fact that we are able to fit the observations at this site with a model that has a lithospheric thickness of 120.7 km suggests that thinner lithospheres may be allowed in models which include lower-mantle transient relaxation.

Further south at Florida the observations do require some elevation of lower-mantle viscosity in order to eliminate the prediction of raised beaches which would otherwise obtain. This is also in accord with results obtained with the Maxwell model¹⁸ and detailed analysis for a wide range of ν_2 values demonstrates that a value of $\nu_2 \approx 3 \times 10^{21}$ Pa s is sufficient for this purpose as well as being allowed by the observations at edge sites like Maine. The main result implied by these analyses of relative sea-level data is the demonstration that the transient Burgher's body model can reconcile glacial isostatic adjustment data to a certain extent, and if $\mu_2/\mu_1 = 0.1$ the model is already behaving very similarly to the Maxwell model, since the lower-mantle value of ν_2 inferred by fitting the model to the data has the same value ($\sim 3 \times 10^{21}$ Pa s) as was previously inferred for ν_1 in the context of the Maxwell analogue. It is rather more difficult to fit all relative sea-level data with the same Burgher's body model than has previously been shown to be the case with the Maxwell model, however, and, as we will proceed to demonstrate, even the small value of $\mu_2/\mu_1 = 0.1$ is too large to accommodate the remaining signatures of the rebound process.

Figure 2 shows observed and predicted J_2 for the three-disk load approximation to the deglaciation history which is detailed in ref. 6, and which contains about 100 m of mean sea-level variation between successive glacial maxima and minima. Again the calculations are based upon the assumption that seven previous 10^5 -yr cycles of glaciation and deglaciation have occurred. The upper horizontal dashed lines on Fig. 2 denote the bounds on J_2 recently inferred by Yoder *et al.*¹, whereas the

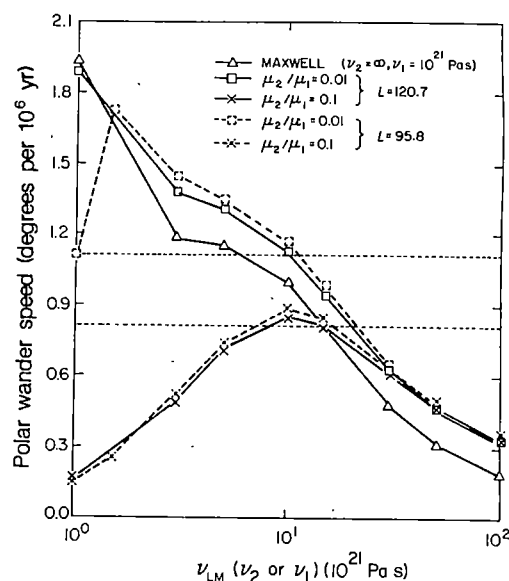


Fig. 3 Predicted polar wander speed as a function of lower-mantle viscosity for the Burgher's body rheologies with $\mu_2/\mu_1 = (0.1, 0.01)$ in which case the lower-mantle viscosity varied as the transient value ν_2 , and for the Maxwell model to which the Burgher's body degenerates in the limit $\mu_2 \rightarrow 0$ in which case the lower-mantle viscosity varied is the steady-state value ν_1 . The solid curves on this figure are predictions for models with lithospheric thickness 120.7 km while the dashed curves are the predictions of models with lithospheric thickness of 95.8 km.

lower lines denote the bounds on the same observations by Rubincam². Predictions are shown as a function of lower-mantle viscosity for three different models. The first model is the Maxwell model which I have used in previous analyses of this datum and the lower-mantle viscosity varied in these calculations is therefore the steady-state viscosity ν_1 . The second and third models are Burgher's body analogues with $\mu_2/\mu_1 = 0.1$ and 0.01 and the lower-mantle viscosity varied in these calculations is the transient viscosity ν_2 since the steady-state value ν_1 is fixed to the value of 10^{23} Pa s on the basis of the results of Richards and Hager¹². As pointed out in ref. 19, the J_2 observation is an especially important one because it is relatively insensitive both to the amount of internal buoyancy in the mantle and to the thickness of the lithosphere. It should therefore deliver a particularly unambiguous constraint upon the contrast in viscosity between the upper and lower mantle. Insofar as the J_2 observation is concerned, the ratio $\mu_2/\mu_1 = 0.1$ is still too large for the Burgher's body to have degenerated to an equivalent Maxwell solid. For $\mu_2/\mu_1 = 0.01$, however, the predictions of the Burgher's body as a function of lower-mantle ν_2 are very close to the predictions of the Maxwell solid as a function of lower mantle ν_1 . Most important, however, in the fact that with $\mu_2/\mu_1 = 0.1$ the lower-mantle value of ν_2 must be near 10^{22} Pa s in order to fit the observed J_2 . This is not compatible with the requirements of the sea-level data discussed previously (particularly those at edge sites like Maine), so that in the context of the Burgher's body $\mu_2/\mu_1 \ll 0.1$ seems to be required. Only if the transient model satisfies the asymptotic condition and thus acts effectively as a Maxwell model is such a model acceptable. This is further confirmed by the second rotational datum.

Figure 3 shows an analogous comparison to that in Fig. 2 but for the speed of polar wander predicted by the three ice cap model⁶. As discussed elsewhere^{6,7}, this is a particularly intricate calculation to perform as the forced polar wander involves a complex interplay between rotational and isostatic adjustment effects. The horizontal dashed lines on Fig. 3 represent the observed speed of polar wander of 0.95 ± 0.15 deg per 10^6 yr. The predicted polar wander direction does not depend at all on the rheology⁶. Also shown for comparison purposes, are results for the two Burgher's body rheologies with a reduced lithospheric thickness of 95.6 km which are shown as the dashed line.

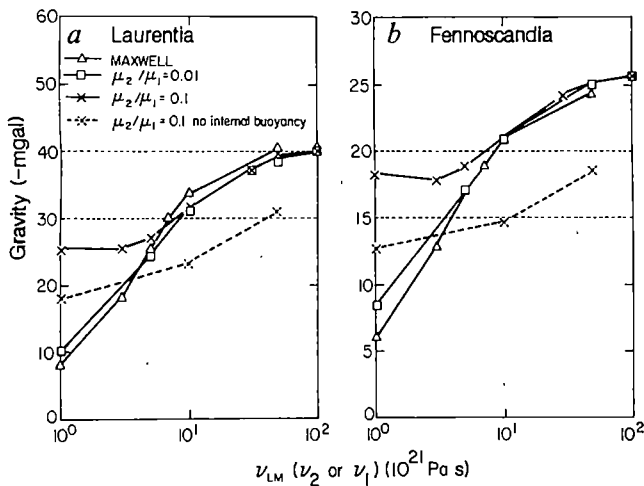


Fig. 4 Predicted peak free-air gravity anomalies at the centres of Laurentia (a) and Fennoscandia (b) as a function of lower-mantle viscosity in the Burgher's bodies with $\mu_2/\mu_1 = (0.1, 0.01)$ in which cases the lower-mantle viscosity varied as the transient value ν_2 , and in the Maxwell analogue to which the Burgher's body converges in the limit $\mu_2 \rightarrow 0$, in which case the lower-mantle viscosity varied as the steady-state value ν_1 . The elastic basic state used in these calculations is the one in which there are density increases of 3.8% and 6.2% at 420 km and 670 km depth respectively. Also shown on these separate plates for the dashed lines are the predicted free-air gravity anomalies for the Burgher's body analogue with $\mu_2/\mu_1 = 0.1$ using an elastic basic state in which the mantle contains no internal buoyancy. Clearly, even in the Burgher's body case considerable internal buoyancy appears to be required to fit the observed free-air anomalies but perhaps somewhat less than for the Maxwell model.

Again, the results for $\mu_2/\mu_1 = 0.01$ are close to the predictions of the Maxwell model to which the Burgher's body degenerates in the limit $\mu_2 \rightarrow 0$. For the transient model with $\mu_2/\mu_1 = 0.1$, however, the polar wander speed is sharply reduced, so much so that it cannot match the observation whatever the value of lower-mantle ν_2 . Indeed, only for the high contrast model with $\nu_2 = 10^{22}$ Pa s is it barely within a standard error of the observed speed of 0.95 deg. per 10^6 yr. This datum also requires large viscosity contrast with $\mu_2/\mu_1 = 0.1$, if it can fit the data at all, and therefore is ruled out by the sea-level data, particularly those at edge sites which display a non-monotonic history of postglacial sea-level variation. The predictions of this signature of the response for the model with thinner lithosphere and $\mu_2/\mu_1 = 0.01$ reveals the same sensitivity as previously demonstrated²⁰ for the Maxwell model. For the larger value of $\mu_2/\mu_1 = 0.1$, however, this sensitivity is absent. It is clearly because of this sensitivity to lithospheric thickness variations that the Maxwell and $\mu_2/\mu_1 = 0.01$ models can be made to fit the observed polar wander speed datum with the small viscosity contrast required by sea-level observations.

Figure 4 shows peak present-day predicted free-air gravity anomalies at the centre of the Laurentian and Fennoscandian disk loads as a function of lower-mantle viscosity for the Maxwell model and for the Burgher's bodies with $\mu_2/\mu_1 = 0.1$ and 0.01 which degenerate to it in the limit $\mu_2 \rightarrow 0$. Inspection of these comparisons again shows that the predictions of the Burgher's body with $\mu_2/\mu_1 = 0.01$ are almost identical to those of the Maxwell model for all values of lower-mantle viscosity. For $\mu_2/\mu_1 = 0.1$, however, the present-day free-air gravity

anomalies predicted by the Burgher's body model are considerably enhanced at low values of ν_2 , implying that one effect of transient lower-mantle rheology may be to allow one to fit the observed anomalies with much lower viscosity contrasts than would otherwise be required. In fact if $\mu_2/\mu_1 = 0.1$ were acceptable on other grounds, which we have shown to be unlikely, one could fit the observed anomaly in Fennoscandia without any viscosity contrast at all and the contrast required in Laurentia would be somewhat reduced. The dashed lines on Fig. 4 show the size of the free-air gravity anomaly which would be expected in these two regions if the mantle contained no source of internal buoyancy within its volume. These predictions are shown only for the Burgher's body with $\mu_2/\mu_1 = 0.1$ and demonstrate that even if this value of μ_2/μ_1 were acceptable, the influence of transient rheology would not completely remove the requirement established previously for the Maxwell model that the density discontinuities at 420 km and/or 670 km depth in the mantle be capable of inducing a buoyancy force when they are deflected from their equilibrium positions by the ice sheet loading process. Peltier²¹ and Peltier *et al.*¹⁸ have provided detailed discussions of the importance of this observation from the point of view of several geodynamic processes including that of mantle convection.

Transient rheology and internal buoyancy

The new calculations described above have reinforced the results of recent analyses based on the suggestion of Weertman¹⁰ that the lower-mantle viscosity to which postglacial rebound is sensitive may be a transient rather than a steady-state value. They do also establish, however, that only transient rheologies in which the elastic defect is very large are candidate rheologies which are allowed by the isostatic adjustment data. Since such rheologies operate essentially as Maxwell rheologies with an effective viscosity $\nu_{\text{eff}} = \nu_1 \nu_2 / (\nu_1 + \nu_2)$, it appears that there is very little to be gained in using the more complicated Burgher's body rheology of Peltier *et al.*¹⁵ to describe isostatic adjustment processes. One simply thereby adds additional parameters to the model which are not required by the data. One extremely important implication of the results obtained here is that introduction of transient relaxation into the lower mantle cannot, apparently, completely relieve the requirement that the internal mantle density stratification be able to deliver some buoyant restoring force when it is deformed in the isostatic adjustment process. Without such internal buoyancy in the mantle it is apparently not possible to reconcile observed free-air gravity anomalies with the same viscoelastic model as is required by relative sea-level observations. As discussed in ref. 21, this point is important since it bears on the question of the chemical stratification of the mantle and/or on the extent to which mantle phase transitions behave in a univariant fashion.

In order to make further progress in constraining the extent to which transient relaxation may be required to explain isostatic adjustment observations, we clearly need an independent re-analysis of the conclusions of Richards and Hager¹² concerning the implications of observed isostatic geoid anomalies. If it could be shown that their slab scale anomalies did not in fact require large viscosity stratification, this would demonstrate that no significant transient component of the mantle relaxation spectrum in fact exists. By combining in this way both long-timescale and short-timescale phenomenology which are sensitive to the relaxation process, we shall eventually be able to refine considerably our understanding of the physical mechanisms by which relaxation actually proceeds.

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Structure of the protein subunits in the photosynthetic reaction centre of *Rhodopseudomonas viridis* at 3 Å resolution

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The molecular structure of the photosynthetic reaction centre from Rhodopseudomonas viridis has been elucidated using X-ray crystallographic analysis. The central part of the complex consists of two subunits, L and M, each of which forms five membrane-spanning helices. We present the first description of the high-resolution structure of an integral membrane protein.

The first steps in photosynthesis are mediated by protein-pigment complexes in the photosynthetic membrane. Light energy is absorbed primarily by light-collecting complexes and transferred to reaction centres where it is used with a very high quantum efficiency to transport electrons across the membrane. The reaction centre from the purple bacterium *Rhodopseudomonas viridis* consists of the four protein subunits L, M, H and c-type cytochrome¹ with four covalently linked haem groups². Other prosthetic groups associated with the reaction centre are four bacteriochlorophyll *b* (BChl-*b*), two bacteriopheophytin *b* (BPh-*b*), two quinones, one non-haem ferrous iron and carotenoid(s)¹. Apart from the cytochrome, which has been found as an integral constituent of reaction centre complexes only in some bacteria, it is generally accepted that reaction centres of purple bacteria are organized in a very similar way. This can be concluded from the subunit composition, from homology between the amino-acid sequences of the L and M subunits, from chromophore composition and from similar spectroscopic and electron-transfer properties (for reviews see, for example, refs 3–7).

The reaction centre from *Rps. viridis* was the first to be crystallized⁸; the photochemical activity of the crystalline material has been demonstrated⁹. An X-ray structure analysis of these well-ordered crystals allowed the calculation of an electron-density map at 3 Å resolution—an atomic model of the prosthetic groups was deduced from this map¹⁰. The arrangement of the pyrrole ring systems of BChl-*b* and BPh-*b* molecules in the central region of the reaction centre (assumed to consist of the subunits L and M) shows an approximate 2-fold symmetry with two closely associated BChl-*b*s near the symmetry axis. These two molecules form the 'special pair' postulated from the results of spectroscopic investigations¹¹. The special pair is symmetrically in contact with two BChl-*b* molecules, each of which has a BPh-*b* as a neighbour. These chromophores form two branches that are apparently suitable as electron pathways. The right-hand side branch (see Fig. 4 in ref. 10) ends with a quinone close to the non-haem iron located on the local symmetry axis. The second quinone and the carotenoid are either not present or disordered in the crystal. The four haem of the cytochrome show a roughly linear arrangement with one haem near the special pair¹⁰.

This chromophore model correlates well with the electron-transfer steps determined from spectroscopy¹². After absorption of light an electron is transferred from the excited primary electron donor (the special pair), possibly via a BChl-*b*¹³ to an intermediate acceptor (BPh-*b*), and further to a primary quinone acceptor (Q_A or tightly bound quinone). Q_A reduces a secondary quinone acceptor (Q_B or loosely bound quinone). Q_B, when fully reduced and protonated, is commonly assumed to leave the reaction centre, and to be replaced from a quinone pool in the membrane¹⁴. The oxidized special pair is reduced by the cytochrome.

Here we report further interpretation of the electron-density map. We describe the folding of the polypeptide chains of the protein subunits in the reaction centre and the spatial relation between protein and prosthetic groups. With this structure we can present the first model of an integral membrane protein determined at nearly atomic resolution.

Electron-density map

The reaction centre crystals belong to the space group P4₃2₁2 with unit cell constants of $a = b = 223.5 \text{ Å}$, $c = 113.6 \text{ Å}$, and contain one centre per asymmetrical unit^{8,10}. The electron-density map at 3 Å resolution was calculated with phases from multiple isomorphous replacement using five heavy-atom derivatives; this map was improved further by solvent flattening¹⁵. A detailed report on data collection, map calculation and arrangement of prosthetic groups is described elsewhere¹⁰.

The chromophore model facilitated the identification of the protein subunits of the reaction centre. The haem groups are evidently bound to the cytochrome subunit; BChl-*b*, BPh-*b*, non-haem iron and quinone are known to be associated with the subunits L and M in reaction centres from purple bacteria^{4,16,17}. Available amino-acid sequences of 46, 30 and 28 residues¹⁸ at the amino-termini of the subunits L, M and cytochrome, respectively, could be located in the map. The H subunit was identified on the basis of a partial gene sequence corresponding to 68 residues from the amino terminus.

For model building of the protein subunits a minimap (scale 2 mm Å⁻¹), and interactive display systems (Vector General 3400 and Evans & Sutherland PS330) driven by the program FRODO¹⁹ were used. Optimum fit of helices and large side chains to the density was achieved with the real-space refinement option in FRODO²⁰. Segments with unknown amino-acid sequence were built as polyalanine. Residue numbers were prefixed with the letters L, M, C and H, respectively, for distinction between subunits.

The good quality of the map allowed unambiguous chain tracing even in the absence of amino-acid sequence information. Recently the complete amino-acid sequences of the subunits H²¹, L and M (H.M., K. A. Weyer, H. Gruenberg, I. Dunger, D. Oesterhelt and F. Lottspeich, in preparation) became available from the corresponding gene sequences and were incorporated into the model. The amino-acid sequence of the cytochrome, presently being investigated with protein-chemical methods (K. A. Weyer, H.M. and F. Lottspeich, unpublished data), is still incomplete. However, peptide sequences resulting from this work could be located in the electron density and used for model building. Currently, these pieces add up to ~60% of the residues. The absence of a contiguous sequence for the cytochrome leads to an uncertainty in the residue numbering for this subunit caused by possible erroneous omission or insertion of residues during chain tracing.

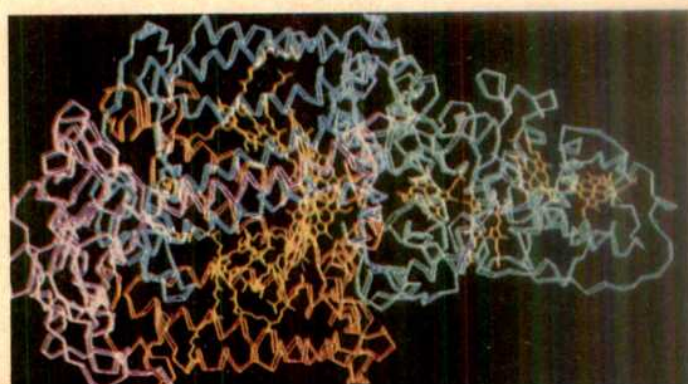
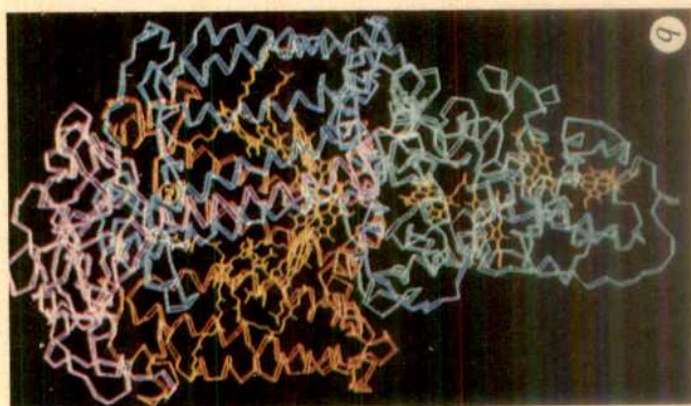
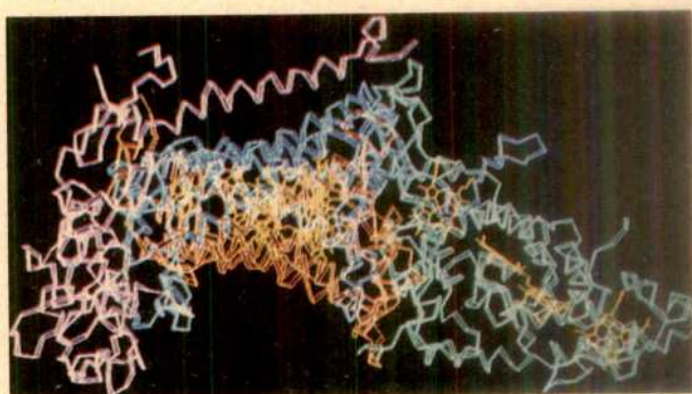
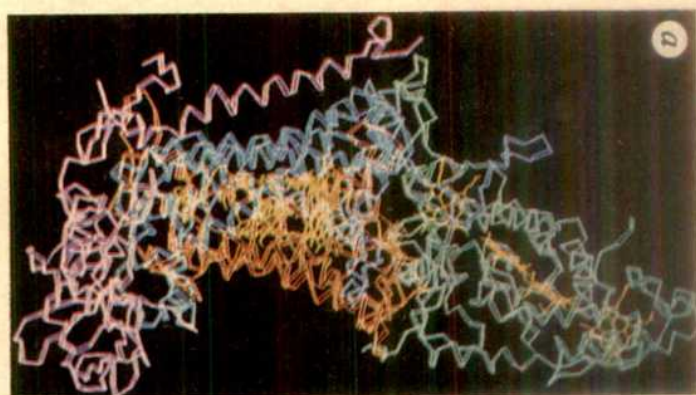
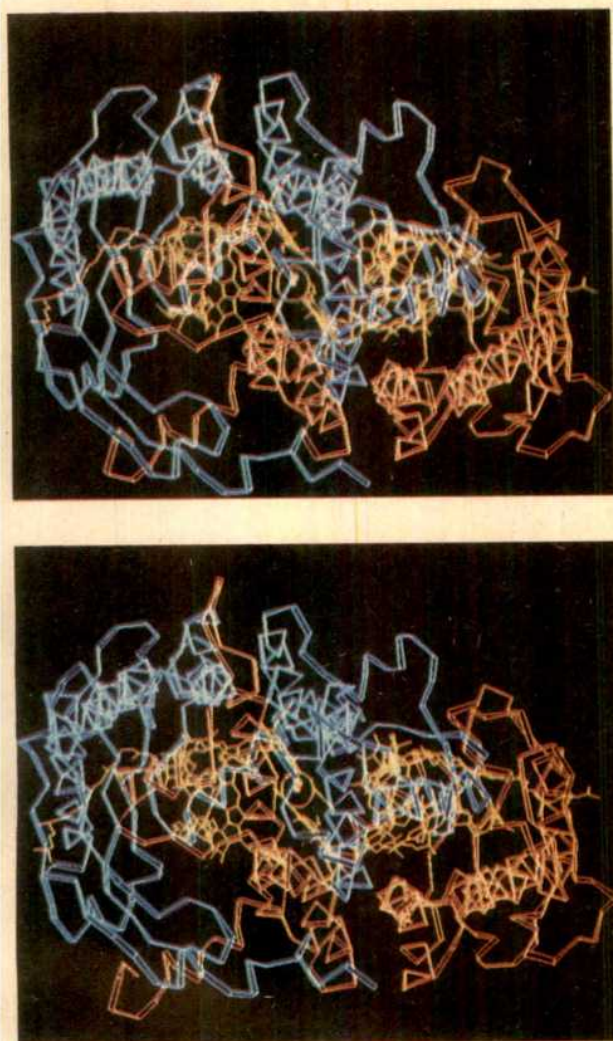


Fig. 1 (Parts *a*, *b* above.) Stereo drawing of the complete reaction centre complex in two orientations. Panel *a* is rotated with respect to *b* by 90° around a vertical axis. Green, cytochrome; orange, L; blue, M; purple, H; yellow, prosthetic groups. Figs 1-5 were produced by a computer program written by A. M. Lesk and K. D. Hardman³⁰.

Fig. 3 (Left) Stereo drawing of the L-M complex and associated chromophores viewed along the L-M local diad. Protein chains are represented as ribbons. Colours as in Fig. 1.

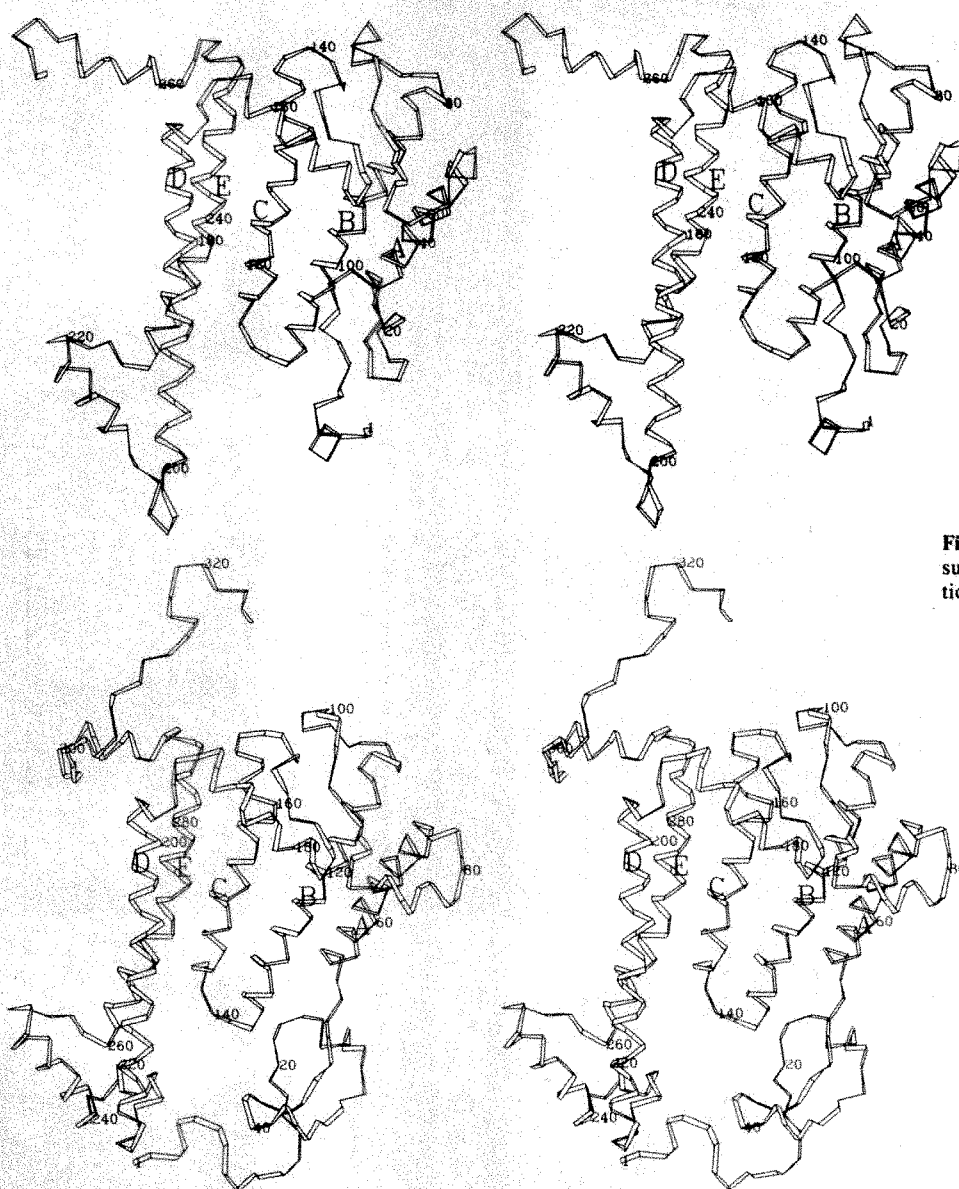


Fig. 2 Stereo drawings of *a*, subunit L; *b*, subunit M represented as ribbons in identical orientations. Transmembrane helices A-E are labelled.

A crystallographic *R*-value ($R = \sum ||F_{\text{obs}}| - |F_{\text{calc}}|| / \sum |F_{\text{obs}}|$) was calculated when the model contained ~70% of the correct sequence. For 46,658 reflections between 7- and 3-Å resolution we obtained $R = 0.359$; this value is exceptionally low for a protein model before crystallographic refinement.

The reaction centre complex

The course of the polypeptide chains and the location of the prosthetic groups of the centre are shown in Fig. 1. The central part consists of the subunits L and M which bind the BChl-*b*, BPh-*b*, the quinone and the non-haem iron. The cytochrome subunit is bound to a flat surface of L-M close to the special pair of BChl-*b*. A large contact area and the carboxy-terminal arm of the M subunit are responsible for the tight binding of the cytochrome to L-M. The major part of the H subunit is in contact with the other flat surface of the L-M complex that is close to the binding sites of the quinone and the non-haem iron. The amino-terminal segment of H forms a helix extending from the cytochrome side along the surface of the M subunit. The longest dimension of the reaction centre complex is ~130 Å between the tips of cytochrome and H subunit.

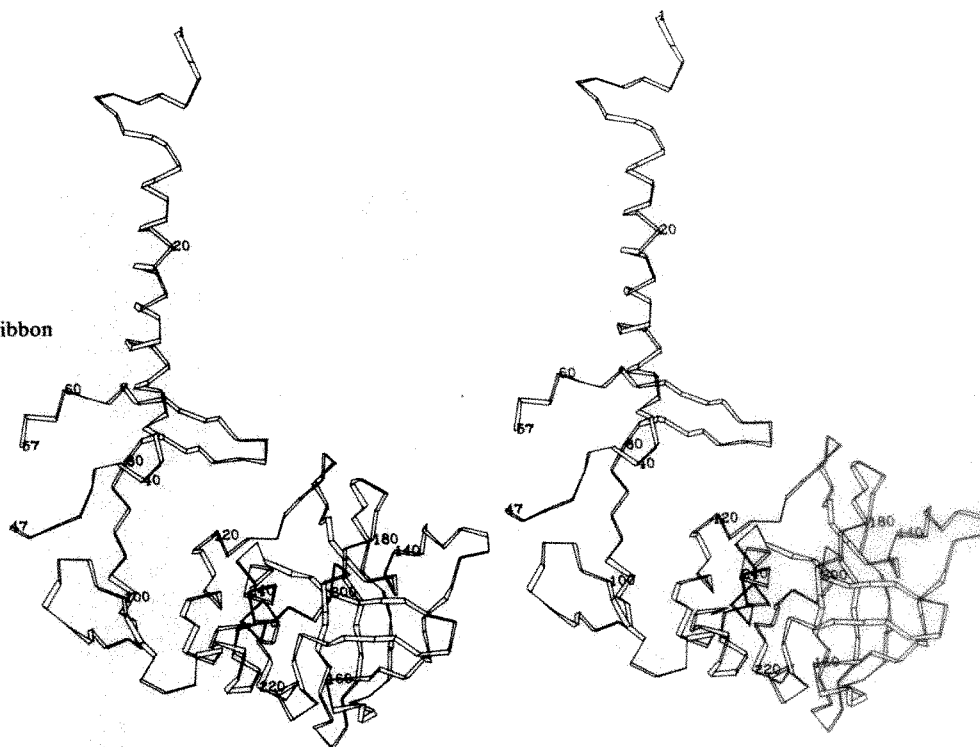
In our electron-density map there is no clear indication of the presence of ordered lipid or detergent that would provide direct evidence on the position and orientation of the membrane relative to the reaction centre complex. However, the highly hydrophobic surface of the central region of the centre indicates that this part (that is, the subunits L and M and the amino-

terminal helix of H) spans the membrane. The same conclusion follows from the fact that with electron transfer from the special pair to the quinone(s) a charge separation across the membrane takes place. Recently, the amino-terminus of the L-subunit was localized at the cytoplasmic side of the photosynthetic membrane from *Rhodospirillum rubrum*²². Assuming the same orientation for the reaction centre from *Rps. viridis*, this means that the cytochrome is located at the periplasmic side, and the major part of the H subunit is at the cytoplasmic side of the membrane.

Subunits L and M

Subunit structure. Although L and M differ in length by 50 residues (L, 273 residues; M, 323 residues), their folding is generally very similar. Using the method of Rossmann and Argos²³, 219 C α -positions of M could be rotated onto corresponding positions of L to an r.m.s. distance of 1.25 Å. The rotation axis is defined by the polar angles $\phi = 43^\circ$, $\Psi = 39.4^\circ$, $\kappa = -179.3^\circ$ (where ϕ is the angle between the rotation axis and *y*-axis; ψ is the angle between projection of the rotation axis on *x-z* plane and *x*-axis; and κ is the rotation angle). At the present level of accuracy this L-M local diad is virtually identical to the local diad relating ring atoms of BChl-*b* and BPh-*b* ($\phi = 43^\circ$, $\psi = 37.6^\circ$, $\kappa = -177.1^\circ$)¹⁰. Figure 2 shows ribbon drawings of subunits L and M in the same orientation. Outstanding structural features of both subunits are five long helical regions (mostly α -helix) numbered A-E (LA-LE in the L subunit,

Fig. 4 Stereo drawing of subunit H (ribbon representation).



MA-ME in the M subunit). They consist mainly of hydrophobic residues, many of which are exposed to the environment. Therefore, we assume that these helices span the membrane; their lengths range between 24 and 30 residues (A, L32-L55, M52-M78; B, L84-L112, M110-M139; C, L115-L140, M142-M167; D, L170-L199, M197-M225; E, L225-L251, M259-M285). The helices are arranged side by side in a concave layer in the order A, B, C, E and D, so that helices C and E are neighbours and run approximately parallel, whereas all other neighbouring helix pairs within a subunit run roughly antiparallel. The angles between the helix axes and the L-M local diad are $<25^\circ$ for the helices A, B, C and E and $\sim 38^\circ$ for helix D.

On both sides of the helical region the remaining segments of L and M form two flat surfaces in intimate contact with the subunits cytochrome and H, respectively. The surface in contact with the H subunit consists of the amino-terminal segments and the helix connections D-E; helix connections B-C are short turns which also come close to this surface. Helix connections A-B and C-D and carboxy-terminal chain segments contribute to the cytochrome contact surface. Apart from as the transmembrane helices, further segments with helical conformation are located in both contact surfaces: L208-L220, M36-M42, M240-M255 at the H contact surface and L149-L164, L259-L269, M80-M88, M178-M192 and M281-M300 at the cytochrome contact surface. Only at the amino termini of L and M two-stranded antiparallel β -sheets are found comprising segments L24-L26, L29-L31 and M27-M35, M44-M51, respectively.

Major structural differences between L and M are observed at or near the contact surfaces. From the amino terminus to the start of transmembrane helix A, M is 20 residues longer than L; these additional residues mainly contribute to the two-stranded antiparallel β -sheet at the H contact surface. The helix connection D-E of M, which is also at the H contact surface, is seven residues longer than in L. At the cytochrome contact surface the helix connection A-B is seven residues longer in M than in L. From the end of helix E to the carboxy terminus M is 16 residues longer than L; the additional residues of M form the arm that folds along the surface of the cytochrome, and appears to contribute to the tight binding of this subunit in the reaction centre complex.

L-M association. The subunits L and M cohere predominantly at or near the H contact surface. Helices D, E and the C-D connection of one subunit intercalate between helices C and E

in the other subunit (see Fig. 3). Helices LD, LE, MD and ME come close to the L-M local diad, and are in contact with each other (see Fig. 1). The non-haem iron is bound between these four helices, each of which contributes one ligand to the iron. Further L-M contacts at the H contact surface are contributed by the helix connections D-E and by the amino-terminal segments (Fig. 3).

Near the cytochrome contact surface a gap opens up between helix pairs LD, LE and MD, ME to accommodate the special pair on the L-M local diad. Helices C and E are bent away from the L-M local diad near the cytochrome contact surface, and so increase the space available for the accessory BChl-*b* and the BPh-*b* molecules. Both L and M contribute one half of the cytochrome contact surface with apparently weak lateral contacts between these halves. At the intersection of the L-M local diad and the cytochrome contact surface the side chain of residue Tyr L162 is located between the closest haem group and the special pair; it may be involved in electron transfer.

With its two only partially overlapping layers of transmembrane helices the L-M complex has a remarkably non-globular structure in which the surface area accessible from the environment appears to be significantly larger than in globular water soluble proteins. Its shape suggests that the contact surfaces are parallel and that the L-M local diad is perpendicular to the membrane plane, in agreement with chromophore orientations determined optically in oriented *Rps. viridis* cells^{24,25}.

Binding of prosthetic groups in L-M. As reported previously¹⁰, protein side chains are bound to the Mg^{2+} ions of BChl-*b*; all four BChl-*b* ligands are histidines as most BChl-*a* ligands found in the BChl-*a* protein from *Prosthecochloris aestuarii*^{26,27}. The BChl-*b*s are designated BC_{LP} , BC_{MP} , BC_{LA} and BC_{MA} , where L and M refer to the ligation and P and A indicate 'special pair' and 'accessory', respectively. The Mg^{2+} ligands of BC_{LP} and BC_{MP} are near the start of the transmembrane helices LD (L173), and MD (M200), respectively. The Mg^{2+} ligands for BC_{LA} and BC_{MA} are in the helix connections C-D (L153 and M180, respectively). The BPh-*b*s are named BP_L and BP_M according to predominant interactions with subunit L or M. Each BPh-*b* binding site is a pocket formed mainly by non-polar residues from both subunits; there are no polar interactions between the protein and the tetrapyrrole nitrogens. BP_L (BP_M) is located near the centres of helices LC, LE (MC, ME) with its ring plane parallel to the axis of helix MD (LD). Accessory BChl-*b* and BPh-*b* are partially accessible from the environment.

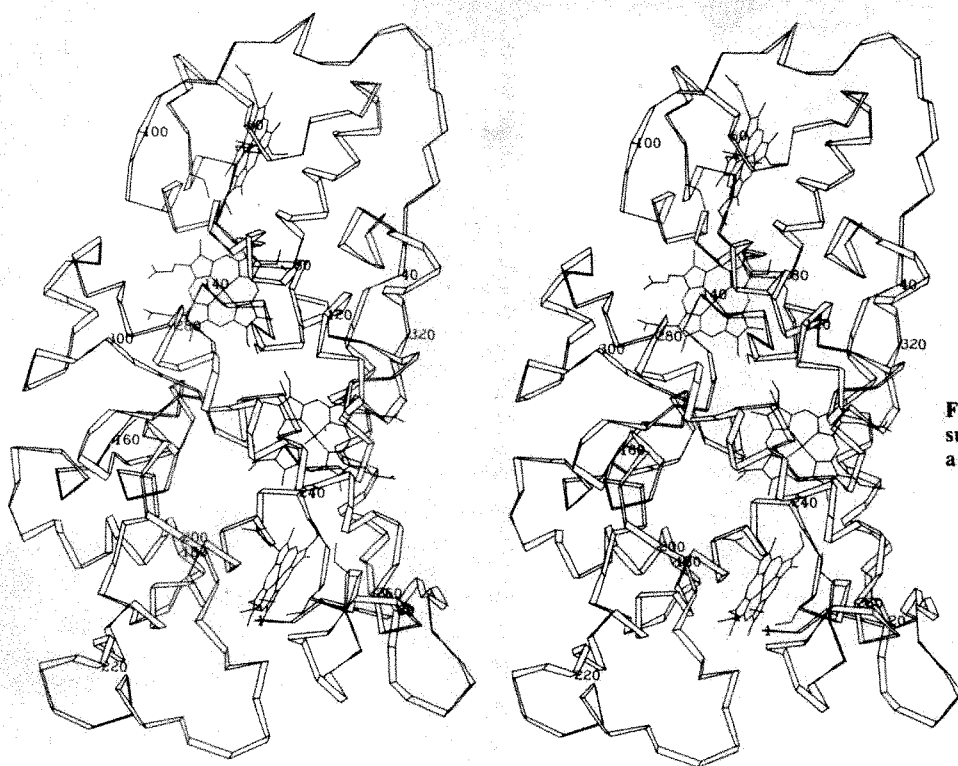


Fig. 5 Stereo drawing of the cytochrome subunit. The protein chain is represented as a ribbon; haem groups and linked cysteine side chains are shown as line drawings.

The non-haem iron is bound by five protein ligands, four histidines and one glutamic acid. The histidines are located in the transmembrane helices LD (L190), LE (L230), MD (M217) and ME (M264), and obey the L-M local 2-fold symmetry. They are arranged approximately in a plane perpendicular to the L-M local diad. The fifth iron ligand, Glu M232, sits on the local L-M diad. The structure near the non-haem iron is in good agreement with conclusions drawn from extended X-ray absorption fine-structure studies^{28,29}.

As reported previously¹⁰, we believe that the quinone identified in the electron-density map is the tightly bound quinone Q_A (menaquinone-9 in *Rps. viridis*³⁰). Q_A is located near BP_L ; its head group is bound exclusively to residues of M in the helix connection D-E with close contacts to His M217 (one of the iron ligands), to the peptide nitrogen of M258 and to Trp M250 whose side chain is nearly parallel to the Q_A head group. Its isoprenoid chain is mainly in contact with BP_L and with residues of L. It was shown that the loosely bound Q_B (ubiquinone-9 in *Rps. viridis*³⁰) can be lost during purification of the reaction centres¹⁴ or during crystallization³⁰. To find the Q_B site, the binding of the competitive inhibitors for Q_B *o*-phenanthroline³¹, terbutryn (a herbicide)³² and of ubiquinone-1 in the crystal was studied by soaking these compounds into the crystals (*o*-phenanthroline, 2 mM for 3 days; terbutryn, 1 mM for 3 days, followed by 0.01 mM for 3 days; ubiquinone-1, 1 mM for 3 days), collecting X-ray data and analysing these data using the difference-Fourier technique. These studies showed binding of the three compounds to a pocket formed near the H contact surface by parts of the transmembrane helices LD and LE, and by the LD-LE connecting segment. Additional binding sites were observed for *o*-phenanthroline (two), and ubiquinone-1 (seven). From these results we conclude that the single common binding site of the three compounds is the Q_B binding site. Only this site, which is related to the Q_A site by the L-M local diad, is close to the non-haem iron as required to explain the broadening of electron-spin resonance lines observed for Q_B^- (ref. 33). The Q_B site is in contact to the iron ligand His L190; the iron itself is located midway between Q_A and Q_B . This arrangement strongly suggests that the iron participates in electron transfer from Q_A to Q_B . However, experiments with iron-depleted reaction centres from *Rps. sphaeroides* showed that electron transfer is only moderately slower in the absence of the iron³⁴.

The binding sites of Q_A and Q_B are in regions of L and M, where both subunits markedly deviate from the L-M local symmetry. Whereas Q_A is completely shielded from the H subunit by the M chain, the probable Q_B binding site is accessible from H through an opening in the H contact surface. These observations correlate the different binding of Q_A and Q_B to structural differences between L and M; they also suggest that one function of H is related to Q_B binding. A more detailed description of the reaction centre structure near the prosthetic groups will be given elsewhere.

The H subunit

With 258 residues H is the smallest subunit of the reaction centre. The course of its polypeptide chain is shown in Fig. 4. The amino-terminus is near the cytochrome contact surface of L-M; residues H1, H2, H3 and H5 are in contact with residues from the cytochrome. Residues H12 to H37 form a helix which runs along the convex side of the surface formed by the transmembrane helices of M, with contacts to helices ME and MD (see Fig. 1). The amino-acid sequence in this helical region up to residue H32 is also primarily hydrophobic; the carboxy-end of the helix (H33-H37) and further on to H39 consists of charged residues (Arg-Arg-Glu-Asp-Arg-Arg-Glu) which interact partly among themselves, and probably also with the polar head groups of membrane lipids *in vivo*.

Altogether we find 11 transmembrane helices in the reaction centre complex, 6 running from the cytoplasmic side to the periplasmic side and 5 running in the opposite direction. The question of whether the dipole moments of these helices can facilitate electron transfer across the membrane, as proposed recently³⁵, requires a detailed inspection of the model and will be discussed elsewhere.

The absence of significant electron density for residues H48 to H56 near the end of the transmembrane helix indicates that this segment is disordered in the crystal. The segment H57-H115 lies on the surface of L-M; residues H66-H70 and H74-H78 form a two-stranded antiparallel β -sheet. The remainder of H is folded into a globular domain. Residues H156-H120 form two layers of β -sheet which enclose a hydrophobic core. Both sheets consist of three strands and show a right-handed twist. In sheet 1 the strands (H162-H174, H179-H187 and H191-H197) are antiparallel; in sheet 2 the middle strand (H207-H210)

is accompanied by an antiparallel strand (H200–H204) and a parallel strand (H156–H159). Near the carboxy-terminus residues H231–H249 form a helix that runs parallel to the H contact surface of L–M.

Little is known about the function of H in the reaction centre complex. Crosslinking studies¹⁶ indicate that H is close to the light-collecting complexes in the membrane; this observation suggests that H has a role in determining the relative orientation of light-collecting complexes and reaction centre. As mentioned above, H may also play a role in Q_B binding—residue H177 (Glu) and its neighbours have access to the Q_B binding pocket in subunit L. They could be involved in donating protons to the photochemically reduced Q_B . This is in accord with experimental results on L–M complexes of *Rps. sphaeroides*³⁶ which show abnormal electron transfer from Q_A to Q_B in the absence of H.

The cytochrome

The present model of the cytochrome subunit contains 333 residues. Figure 5 shows the course of the polypeptide chain and the location of the haem groups. Near the amino-terminus, residues C7–C11 and C20–C23 form an antiparallel β -sheet that lies on the L half of the cytochrome contact surface. From there the chain proceeds to the tip of the subunit (around C50); residues C25–C37 form a helix. In the following bulk of the subunit four recurring structural motifs can be observed: a helix runs parallel to the ring plane of each haem group (residues C67–C82, C102–C119, C222–C240 and C262–C286, respectively); the haems are covalently attached near the carboxy termini of these helices where the chains turn back towards the haems. The electron density strongly indicates that these covalent links are thioether bridges, and that the sequences of the haem binding segments are Cys–X–X–Cys–His as in other *c*-type cytochromes; the His residues are the fifth ligands to the haem irons. The haems are numbered according to the order of attachment to the protein; for haem 1 the first covalent link is to residue C87, haem 2, C132, haem 3, C244 and haem 4, C305. All haem irons appear to be six-coordinated; the sixth ligands to the irons of haem 1, 2 and 3 are located in the helices associated with the haems (residues C74, C110 and C233, respectively). The sixth ligand to the haem 4 iron (C124) is in the segment between the helix parallel to haem 2 and the haem 2 attachment site. According to the electron density the sixth ligands to the haem irons could be histidines or methionines.

As reported previously¹⁰, the haem groups are arranged with an approximate 2-fold symmetry: pyrrole ring atoms of haems 3 and 4 can be rotated onto those of haems 1 and 2 with polar angles $\phi = 117.2^\circ$, $\psi = -70.2^\circ$ and $\kappa = -176.5^\circ$ to an r.m.s. distance of 0.41 Å. Haems 1 and 2 and haems 3 and 4 are related by improper rotations ($\sim 105^\circ$) with large screw components (~ 12 Å). Using the same method as for the L–M comparison²³, 66 C α positions from the region C226–C315 could be rotated onto corresponding C α positions from between C67 and C142 to an r.m.s. distance of 1.1 Å. The polar angles of this rotation are $\phi = 116.7^\circ$, $\psi = -68.9^\circ$ and $\kappa = -177.8^\circ$, very close to those for the haem rotation. This internal 2-fold symmetry includes about one third of the cytochrome subunit. More extended structural homologies with 2-fold symmetry in protein molecules have been reported for rhodanese³⁷ and γ -crystallin³⁸. The intramolecular symmetry is broken in the segment connecting the binding sites of haem 2 and 3; this segment contributes to the contact of cytochrome to L–M. The carboxy-terminal segment ends near the helix associated with haem 2. Haem 1 is accessible from the environment at its pyrrole rings I and IV; haem 2 appears to be almost completely buried; and haems 3 and 4 are accessible at their propionic acid groups. Pyrrole rings I and IV of haem 3 are closest to the special pair of BChl-*b* in the L–M complex. Apparently, protein folding in the cytochrome subunit of the reaction centre is different from both cytochromes C2 and cytochromes C3 with known three-dimensional structure (see ref. 39 for review).

Structure of related proteins

Reaction centres from other purple bacteria. Amino-acid sequences are known for the reaction centre subunits L, M and H from *Rps. capsulata*⁴⁰, and for L and M from *Rps. sphaeroides*^{41,42}. The sequences of L and M from *Rps. viridis* show between 50 and 60% homology with those of the corresponding subunits from the other two bacteria. On the basis of hydrophobicity five transmembrane helices each were postulated^{40–42} in segments of L and M homologous with the transmembrane helix regions in our model. All the ligands to the BChl and to the non-haem iron described above are also conserved, and the polypeptide segments connecting these ligands have the same length in all three organisms. These observations clearly indicate that the structures of L and M from *Rps. capsulata* and *Rps. sphaeroides*, and the arrangement of BChls and non-haem iron are very similar to those found for *Rps. viridis*. Significant differences in polypeptide chain length occur exclusively at the carboxy-termini where L from *Rps. viridis* is shorter by 8 residues, and M is longer by 17 residues. These additional residues of M are in contact with the cytochrome in *Rps. viridis*: the reaction centres of *Rps. capsulata* and *Rps. sphaeroides* lack a permanently bound cytochrome subunit.

For the H-subunits from *Rps. capsulata* and *Rps. viridis* the sequence homology is 37% (ref. 21). Common features of these two H-subunits include a hydrophobic region near the amino terminus which corresponds to the transmembrane helix of H in our model. In addition, many of the residues involved in the contact between H and L–M are conserved in both organisms, so that structural similarity can also be expected between the H subunits from *Rps. capsulata* and *Rps. viridis*.

Photosystem II. Recently, amino-acid sequences of polypeptides from photosystem II reaction centres^{43–47} have been reported. Sequence homologies were found between L and M of purple bacteria and the D1 polypeptide (also referred to as Q_B protein or herbicide binding polypeptide) from photosystem II of higher plants^{40,42} and between D1 and D2 (ref. 45) which is also a constituent of photosystem II. In these plant proteins, histidines which correspond to the ligands of the special pair, and to those of the non-haem iron in our model are conserved. Even a Trp residue corresponding to M250, which forms part of the Q_A binding site in reaction centres of purple bacteria, can be found in the D2 proteins. These observations corroborate our recent proposal⁴⁸ that proteins D1 and D2 form the core of the photosystem II reaction centre, similar to the subunits L and M in purple bacteria. Based on functional and sequence homology we believe that D1 and D2 span the membrane five times, contrary to a proposed seven-helix structure⁴⁹, where the helix connections A–B and D–E were probably misinterpreted as transmembrane helices.

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Identification of the signal molecules produced by wounded plant cells that activate T-DNA transfer in *Agrobacterium tumefaciens*

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We show here that Agrobacterium tumefaciens virulence (Vir) gene expression is activated specifically by the plant molecules acetosyringone (AS) and α -hydroxyacetosyringone (OH-AS). These molecules induce the entire vir regulon in Agrobacterium as well as the formation of T-DNA intermediate molecules. AS and OH-AS occur specifically in exudates of wounded and metabolically active plant cells and probably allow Agrobacterium to recognize susceptible cells in nature.

SOIL bacteria often form specialized interactions with plant cells. Usually a particular bacterium interacts with only a few species and/or types of plant cells^{1,2}. A primary step in the formation of a bacterial/plant interaction is the detection by the bacterium of the appropriate susceptible plant cell. This recognition then triggers the activation of the bacterial genes whose products direct the development and/or maintenance of the interaction. The soil is a complex biological and chemical environment, and the signals that mediate the detection of a specific plant cell by a bacterium are unknown.

A. tumefaciens, a soil phytopathogen, genetically transforms dicotyledonous plant cells to cause the neoplastic disease crown gall^{3,4}. Only cells that have been wounded are seen to be susceptible^{5,6}. The bacterium transfers a specific segment of DNA, the T-DNA, from its large (> 200 kilobases, kb) tumour-inducing (Ti) plasmid to the susceptible plant cell, where it becomes integrated into the nuclear genome^{7,8}. In the Ti plasmid, the T-DNA is defined and bounded by identical 25-base pair (bp) direct repeats; only DNA between these T-DNA borders is transferred to the plant genome⁹⁻¹². During co-cultivation with plant cells, independent T-DNA circles are formed in *Agrobacterium*; these molecules arise by a specific recombination between the 25-bp sequences at the ends of the T-DNA and are potential intermediates in the transfer of the T-DNA from *Agrobacterium* to the plant cell¹³.

The Ti plasmid genes required for plant transformation are not contained in the T-DNA but are located in the ~40-kb *vir* region¹⁴⁻¹⁶. Genetic analysis of the *vir* region of the A6 Ti plasmid has shown that it encodes at least six separate complementation groups, *virA*, *B*, *C*, *D*, *E* and *G*, and *pinF* that are organized as a single regulon (ref. 17; S.E.S., in preparation). In the vegetative bacterium only *virA* and *virG*, the *vir* regulatory genes, are significantly expressed; however, when *Agrobacterium* is co-cultivated with plant cells the expression of *virB*, *C*, *D*, *E*,

G and *pinF* is induced to high levels^{17,18}. This activation of *vir* expression by plant cells probably initiates the steps of T-DNA transfer and integration into the plant cell genome. We have shown that *vir* induction and the production of T-DNA circles is mediated by a small diffusible factor produced by actively growing plant cells¹⁷. Here, we purify and establish the chemical identity of this factor, and demonstrate that its production is related to plant cell wounding and that its recognition by the bacterium is a highly specific process. This factor is likely to be the signal that allows *Agrobacterium* to recognize in nature a plant cell susceptible to transformation.

Purification of signal molecules

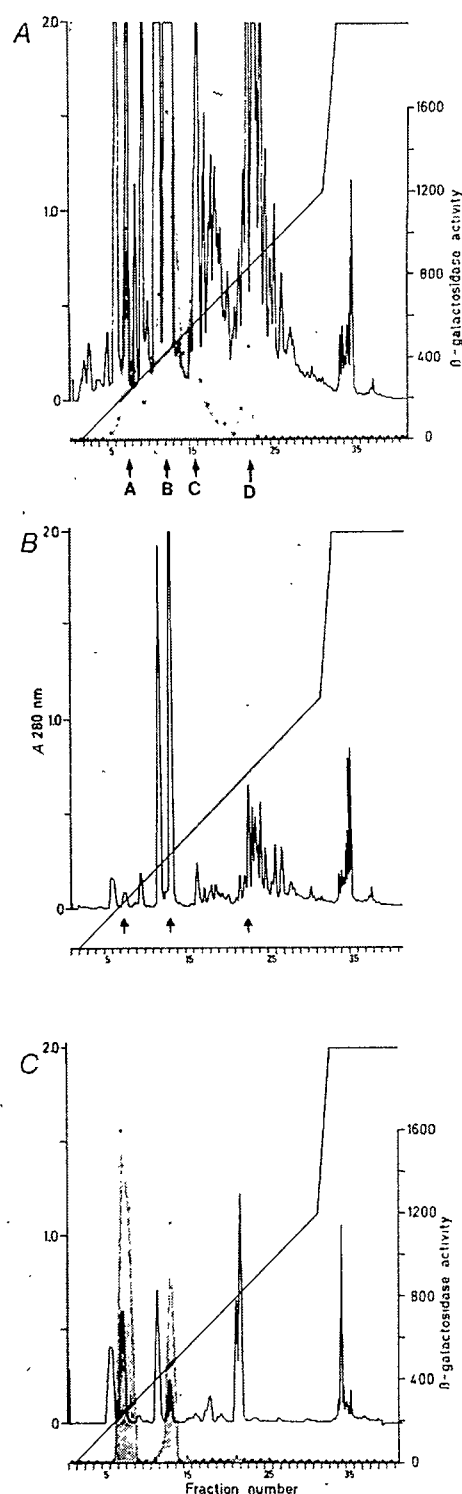
To purify the plant molecule(s) that specifically signals *Agrobacterium* to initiate its interaction with plant cells requires first, an efficient and quantitative bioassay for a primary event in this transformation process, specifically the induction of *vir* gene expression; and second, a starting source of the signal molecule(s). We have previously described an assay for *vir* induction in *Agrobacterium* that used gene fusions between the pTiA6 *vir* loci and the *Escherichia coli lacZ* gene^{17,18}. Briefly, in a bacterium carrying a *vir::lac* gene fusion, the production of β -galactosidase (the *lacZ* gene product) is controlled by the *vir* locus to which *lacZ* has been fused. Thus, the state of expression of the locus can be monitored by measuring the β -galactosidase activity present in the bacterium; increased activity indicates increased *vir* expression. (The relative amount of induced activity reflects the relative amount of *vir*-inducing activity to which the cell has been exposed.) Here we use *Agrobacterium* strain A348(pSM30) (ref. 17) to detect and measure *vir*-inducing activity. This strain contains wild-type pTiA6 and pSM30, a *virB::lac* fusion plasmid, and gives high levels (up to ~100-fold of basal activity) of induced β -galactosidase activity¹⁷.

Fig. 1 RPC/FPLC fractionation of the *vir*-inducing activity in plant-cell exudates. The organic compounds present in plant cell medium conditioned with root culture (*cmr*; A, B), or with leaf disks (*cml*; C), were prepared by chloroform extraction and analysed by RPC/FPLC. The material analysed in A, B and C was prepared from 2 litres *cmr*, 40 ml *cmr* and 50 ml *cml*, respectively. Each sample was fractionated on a C-2/C-18 RPC column eluted with a linear methanol gradient. In A and C, column fractions were bioassayed for *vir*-inducing activity in *Agrobacterium*. The diagonal line represents the elution gradient and the solid line indicates the ultraviolet absorbance measured at 280 nm of the column fractions; shaded curves indicate relative specific units of β -galactosidase activity induced in the *virB::lacZ* tester strain A348(pM30) by the column fractions. The major peaks of *vir*-inducing activity in A are indicated by arrows labelled A, B, C and D. B, Arrows indicate peaks corresponding to peaks A, B and D of A.

Methods. Root culture of *N. tabacum* transformed with *Agrobacterium rhizogenes* A15834 was grown and maintained as described previously¹⁷. Transformed roots were used because they are easy to propagate. Every 72 h the conditioned medium (*cmr*) was removed and stored at -20°C . Leaf disks were prepared from 6-week-old untransformed *N. tabacum* SR1 plants, and 2-g samples of 1.5-cm-diameter disks were incubated in 50 ml MS medium (Murashige and Skoog²⁸ salts, 3% sucrose, supplemented with 0.018% K_2HPO_4 , 0.01% inositol, 0.0001% biotin, pH 5.5) in 150-mm Petri dishes. After 72 h, the conditioned medium (*cml*) was removed and stored at -20°C . The conditioned medium (*cmr* and *cml*) was filtered through 0.22 μm nitrocellulose and the filtrate extracted twice with a 25% volume of chloroform and the pooled chloroform phase was back-extracted with 1 vol. MS medium, rotary-evaporated to dryness and resuspended in 500 μl 20% CH_3OH , 0.1% CH_3COOH for analysis by RPC/FPLC. (Note that initial solvent extraction experiments were performed to determine the solvent partitioning character of the *cmr* and *cml* *vir*-inducing activities. In these experiments 50 ml of the respective conditioned medium was extracted with chloroform; the interphase and chloroform phase were lyophilized and each resuspended in 2 ml MSSP (MS medium supplemented with 12.5 mM sodium phosphate, pH 5.5¹⁷), and the aqueous phase was blown with a stream of air to remove traces of chloroform. Each of these samples was bioassayed for *vir*-inducing activity; only the chloroform phase material contained this activity.) A Pep RPC pre-packed 5 mm \times 50 mm column (HR5/5) containing 6- μm silica particles with C-2 and C-18 alkyl side chains (Pharmacia) was pre-equilibrated with 10% CH_3OH , 0.1% CH_3COOH . The column was run at a flow rate of 0.7 ml min^{-1} using a Pharmacia FPLC system equipped with the LCC500 chromatographic programmer. A single-path ultraviolet monitor was used to monitor absorbance at 280 nm. The column was eluted with a linear gradient of 10–60% $\text{CH}_3\text{OH}:\text{H}_2\text{O}$ (v/v), 0.1% CH_3COOH . We collected 40 1.4-ml (2-min) fractions; 140- μl aliquots (A) or the entire fractions (C) were lyophilized, resuspended in 1.5 ml MSSP and bioassayed for *vir*-inducing activity. Overnight cultures of strain A348(pSM30) were centrifuged and resuspended in MSSP medium. Material to be tested for *vir*-inducing activity was inoculated with bacteria at 0.1 absorbance unit ml^{-1} at 600 nm cm^{-1} . Incubations were for 10 h at 28°C and 200 r.p.m. Specific units of β -galactosidase activity were determined as described previously^{17,18} and are expressed as U per bacterial cell.

Induction of *vir* expression occurs during co-cultivation of *Agrobacterium* with plant cells, and during incubation of bacteria in plant-cell exudates. We have shown that the medium in which *Nicotiana tabacum* root culture has grown (designated *cmr* for conditioned medium roots) contains substantial amounts of a *vir*-inducing activity¹⁷. This activity stimulates the expression of each of the inducible pTiA6 *vir* loci, and also the formation of T-DNA circular intermediates, indicating that it triggers in *Agrobacterium* the initiation of plant cell transformation. This activity has relative molecular mass less than 1,000; is stable to boiling, freezing, lyophilization, and high and low pH; and is partially hydrophobic, as it is retained by silica C-18 and completely elutes from this matrix with 40% CH_3OH (ref. 17). Here we purify and identify this *cmr* activity.

The above properties suggest that the *cmr* *vir*-inducing activity is composed of one or more small organic molecules; that it completely partitions into the organic solvent chloroform (Fig. 1) confirms this identity, and provided a basis for its purification. The *cmr* *vir*-inducing activity was fractionated by reverse-phase chromatography (RPC) using a high-resolution fast-performance liquid chromatography (FPLC) system. Activity was localized to two major and two minor peaks that had eluted, respectively, with 18 (peak A), 27.5 (peak B), 32



(peak C) and 42% (peak D) CH_3OH (Fig. 1A). Thus, the *cmr* *vir*-inducing activity is represented by at least four distinct compounds. When less material is loaded onto the FPLC column (Fig. 1B), peak B is the major component of the organic fraction of *cmr*. Because of their low abundance, the peak C and peak D activities were not analysed further. The signal molecules in peaks A and B were purified to homogeneity by further RPC fractionation and analysed by gas chromatography/mass spectroscopy (GC-MS) (Fig. 2) and ultraviolet absorption spectroscopy (Fig. 3).

Identification of *vir* inducers

The peak B compound, the major *cmr* *vir*-inducing molecule, is 4-acetyl-2,6-dimethoxyphenol, based on several experimental observations: (1) The GC/MS spectrum of the peak B compound

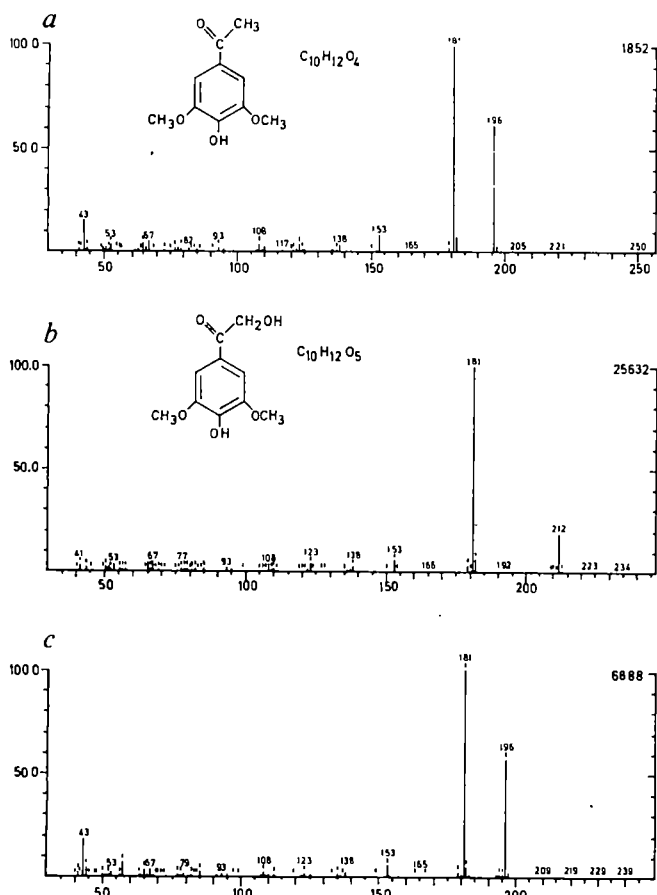


Fig. 2 Mass spectra of *vir*-inducing molecules. The mass spectra of purified peak B compound (Fig. 1), purified peak A compound (Fig. 1) and authentic acetosyringone (Janssen Chimica), are shown in *a*, *b* and *c*, respectively. Vertical axes, relative intensity of the fragment ions; horizontal axes, the mass/charge ratio of the fragment ions. The determined chemical formulae and structures of peak B and peak A (from Fig. 1) are indicated in *a* and *b*, respectively.

Methods. The biologically active compounds present in the peak A and B fractions were purified to homogeneity by RPC/FPLC; fractionation conditions were identical to those described in Fig. 1, except for the column solvent. The fractions containing peak A or peak B (from Fig. 1A) were pooled, lyophilized, resuspended in 5% acetonitrile, 0.1% trifluoroacetic acid (TFA) and injected onto the C-2/C-18 column equilibrated with this buffer. The column was eluted with a linear gradient of 5–30% acetonitrile, 0.1% TFA, and monitored for ultraviolet absorbance at 280 nm and for biological activity. For each sample a single peak was resolved from other minor ultraviolet-absorbing compounds, and a portion of the peak fraction was lyophilized, dissolved in 500 μ l chloroform and evaporated to 40 μ l under a nitrogen stream for GC/MS analysis. Separate portions of each sample were dissolved in CH_3OH for ultraviolet absorption analysis (Fig. 3) and in MSSP for analysis of biological activity. We injected 0.5 μ l of sample into a 0.32 mm \times 25 m Carlo Erba (HRGC) gas chromatography column SF-52, directly coupled to a Finnigan 4000 mass spectrometer. GC fractionation was with a 50–250 $^\circ$ gradient, 5 $^\circ$ min $^{-1}$. Data were collected and processed on a Nova 3 computer (Data General).

(Fig. 2a) indicates a molecule of relative molecular mass (M_r) 196, chemical composition $\text{C}_{10}\text{H}_{12}\text{O}_4$ and general structure acetyl-dimethoxyphenol. (2) Comparison of the ultraviolet absorption spectrum of the peak B compound in CH_3OH with its spectrum in $\text{CH}_3\text{OH}/\text{NaOH}$ (Fig. 3a) indicates that the molecule exhibits a strong absorption shift to longer wavelengths in the presence of NaOH; this base-induced redshift is diagnostic of phenolic compounds in which the phenolic hydroxyl group is *para* (but not *ortho* or *meta*) to a conjugated ring substituent, such as a ketone or allyl group¹⁹. (3) The *p*-hydroxyl derivative, 4-acetyl-2,6-dimethoxyphenol, commonly termed acetosyringone (AS), is commercially available and greatly stimulates *vir*

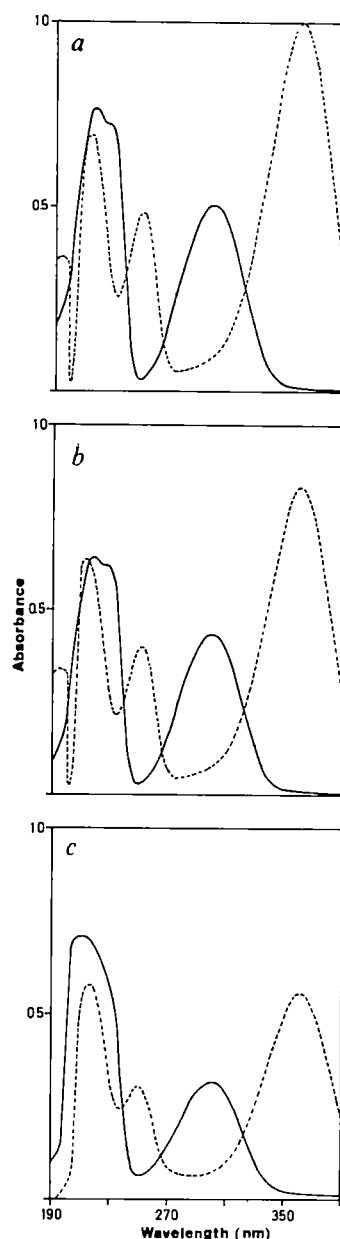


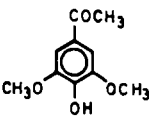
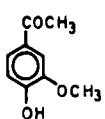
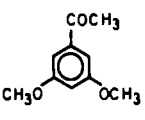
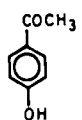
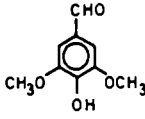
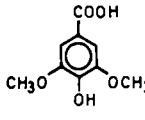
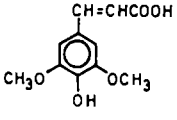
Fig. 3 Ultraviolet absorption spectroscopy of *vir*-inducing compounds purified from *cmr*. Spectra were determined in both CH_3OH (solid line) and $\text{CH}_3\text{OH}/\text{NaOH}$ (dashed line). *a*, Spectra of peak B compound; *b*, spectra of commercially obtained acetosyringone; *c*, spectra of peak A compound.

Methods. Absorption spectra were measured in a Beckman DU-6 spectrophotometer scanning at 60 nm min $^{-1}$. For each compound dried material was resuspended in CH_3OH (HPLC grade, Rathburn Chemicals) to an approximate concentration of 50 μM , and scanned against CH_3OH as a blank solution. The pH of the sample was subsequently adjusted to basic pH by the addition of 1 M NaOH to 20 mM and the sample scanned against $\text{CH}_3\text{OH}/20 \text{ mM NaOH}$. (Note that when the base-adjusted sample of each compound is readjusted to acid pH by adding 1 M HCl to 40 mM, the determined spectrum is equivalent to the original CH_3OH spectrum of the compound (data not shown). Thus, the base-induced redshift of each compound is fully reversible in the conditions used.)

expression in *Agrobacterium* (see below). AS and the purified peak B compound co-elute on RPC/FPLC (data not shown) and their GC/MS spectra are indistinguishable (Fig. 2). Furthermore, their respective ultraviolet spectra, both in CH_3OH and in $\text{CH}_3\text{OH}/\text{NaOH}$, are identical (Fig. 3a, b). (4) The quantitative *vir*-inducing activities of authentic AS and the peak B compound are equivalent. In the experiment described in Fig. 4, the units of induced β -galactosidase activity as a function of concentration of inducing compound were determined for each compound at several concentrations; the respective activity/concentration curves are identical.

The peak A compound, the second major *cmr vir*-inducer, is 4-(2-hydroxyacetyl)-2,6-dimethoxyphenol, termed α -hydroxy acetosyringone (OH-AS), an analogue of AS. This identification is based on the following observations: (1) The GC/MS spectrum of the peak A compound (Fig. 2c) indicates a molecule of M_r 212 whose only significant fragmentation product has M_r 181. The GC/MS spectrum of compound A following trimethylsilylation indicates a mass increase corresponding to two trimethylsilyl groups (data not shown). Thus, compound A contains two hydroxyl groups and has general structure of either (3-propanol)dimethoxyphenol ($\text{C}_{11}\text{H}_{14}\text{O}_4$) or (2-hydroxyacetyl)dimethoxyphenol ($\text{C}_{10}\text{H}_{12}\text{O}_5$). Only the latter structure is consistent with the observation that the peak A compound is more polar than AS, as it elutes before AS on RPC/FPLC

Table 1 Biological activity of derivatives of acetosyringone

a acetosyringone		110	109	93	29
b acetovanillone		80	33	1.9	1.6
c 3,5-dimethoxy acetophenone		ND	1.0	ND	ND
d 4-hydroxy acetophenone		ND	1.1	ND	ND
e syringaldehyde		86	51	2.2	ND
f syringic acid		21	8.6	1.7	ND
g sinapinic acid		104	98	68	18

Acetosyringone (**a**) and six related compounds; acetovanillone (**b**); 3,5-dimethoxy acetophenone (**c**); 4-hydroxyacetophenone (**d**); syringaldehyde (**e**); syringic acid (**f**); and sinapinic acid (**g**), were tested for their ability to induce β -galactosidase in the *Agrobacterium virB::lacZ* strain A348(pSM30). All compounds were purchased from Janssen Chemica, and prepared as 0.1 M solutions in dimethyl sulphoxide. Each compound was serially diluted into MSSP (Fig. 1, legend) medium to 200, 50, 5 and 0.5 μ M (**a**, **b**, **e**, **f**, **g**), or 50 μ M (**c**, **d**). These solutions were inoculated with bacteria at 0.05 absorbance unit ml^{-1} at 600 nm cm^{-1} and incubated at 28 °C, 200 r.p.m. After 12 h, the bacterial β -galactosidase activity in each sample was determined. The data are expressed as activity of the bacteria incubated in the presence of a compound relative to the basal activity present in bacteria incubated without added compound. The basal activity for the pSM30 strain is 10 U. ND, not determined.

(Fig. 1). (2) Comparison of the ultraviolet adsorption spectrum of the peak A compound in CH_3OH with its spectrum in $\text{CH}_3\text{OH}/\text{NaOH}$ indicates that, similarly to AS, this molecule exhibits a strong base-induced redshift (Fig. 3c). Thus, compound A contains a phenolic hydroxyl group *para* to a conjugated ring substituent. Furthermore, the absorption spectra of compound A are almost identical to the equivalent spectra of AS; the respective absorption maxima occur at identical wavelengths (the largest maxima occur at 298 nm in CH_3OH and 355 nm in $\text{CH}_3\text{OH}/\text{NaOH}$), although their relative extinction coefficients are different. These similarities strongly indicate that the peak A compound is very closely related to AS. (3) The quantitative *vir*-inducing activity of the peak A compound is approximately equivalent to that of AS (Fig. 1). As described below, the structure/activity specificity of *vir*-induction indicates that only molecules with similar structure to AS induce *vir* expression to high levels (Table 1).

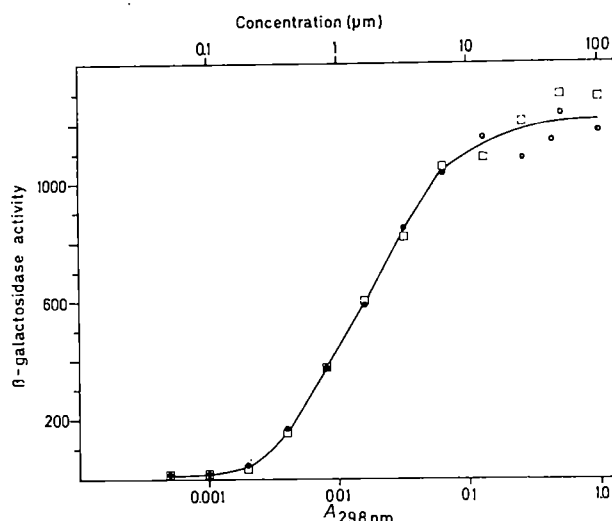


Fig. 4 Comparison of *vir*-inducing activity of purified peak B compound (Fig. 2) and commercially obtained acetosyringone. Vertical axis, β -galactosidase activity (U bacterium $^{-1}$) in the *Agrobacterium virB::lacZ* strain A348(pSM30); lower horizontal axis, relative concentration of purified peak B compound (Fig. 2) or authentic AS (Janssen Chimica) in the inducing medium, measured as absorbance at 298 nm (cm^{-1}). The extinction coefficient of AS at 298 nm is 10,300 (ref. 29); this value was used to calculate the concentration of inducer compound in the inducing medium, indicated by the upper horizontal axis.

Methods. Purified peak B compound and authentic AS were separately dissolved in MSSP to 1.0 absorbance unit ml^{-1} at 298 nm (cm^{-1}), measured against fresh MSSP. Each solution was serially diluted and the resultant samples were inoculated with bacterium at 0.09 absorbance unit ml^{-1} at 600 nm (cm^{-1}). Samples were incubated for 14 h at 28 °C, 200 r.p.m. and the β -galactosidase activity of the bacteria in each sample determined.

Thus, we have identified plant molecules that induce *vir* expression. Our next experiments seek to provide insight into the relationship between these molecules and *Agrobacterium* in nature. For the *Agrobacterium*/plant transformation system to be most efficient, its activation should be limited to the presence of susceptible plant cells. This could be achieved if activation is signalled only by molecules specific to these cells. For instance, such molecules should first, be produced by different types of plant cells, as *Agrobacterium* can transform several different cell types²⁰; second, be specifically synthesized by susceptible plant cells, such as wounded cells; and third, be available to the bacterium in quantities sufficient for efficient activation. The following experiments demonstrate that AS has these properties and that activation of *Agrobacterium* is a highly specific process.

Different plant tissues produce AS/OH-AS

To determine whether AS and OH-AS are specific to roots or whether they are also produced by other plant tissues, we purified and identified the *vir*-inducing activity produced by leaf cells (Fig. 1). Medium in which *N. tabacum* leaves that have been cut into disks have been cultured (*cml*; conditioned medium leaves) contains substantial amounts of inducing activity that completely partitions into chloroform. The RPC/FPLC profile and corresponding bioassay profile of this material (Fig. 1c) show that the *cml* activity fractionates into two peaks that co-elute with OH-AS and AS. Subsequent purification and GC/MS and ultraviolet spectrophotometric analysis of the active molecules in these peaks confirmed that the major *vir*-inducing activity in *cml* is composed of OH-AS and AS (data not shown). Thus, these two compounds are present in the exudates of at least two different plant tissues.

AS and OH-AS are exudate-specific

In the soil, *Agrobacterium* probably detects plant cells through their exudates. To determine whether AS and OH-AS are

exudate-specific we assessed their relative concentrations within the *N. tabacum* leaf disks of Fig. 1C. These disks were extracted with chloroform and the extract was analysed by RPC/FPLC fractionation and corresponding bioassay. The relative concentrations of AS and OH-AS are <0.5% of the organic compounds present in the total leaf disks (data not shown). In comparison, the relative concentrations of these compounds in the *cml* extract is ~25% and greater (Figs 1C, 5A). Thus, AS and OH-AS probably do not leak out of damaged plant cells and are exudate-specific compounds.

Concentration of *vir* inducers in *cmr/cml*

Figure 4 shows the relationship between the concentration of AS (both commercial and *cmr*-purified) and induction of *virB* expression as measured in units of β -galactosidase activity. Under the conditions used, AS at $\geq 10 \mu\text{M}$, and AS at $1.5 \mu\text{M}$, induce maximal (1,200 U) and half-maximal (600 U) expression, respectively. Note that AS induction does not significantly affect cell viability (that is, induction is a non-lethal event), and that concentrations of AS $>200 \mu\text{M}$ are not significantly toxic to the bacteria. Using the dose-response curve of Fig. 4, the concentration of *vir*-inducing compounds in plant-cell exudates relative to AS can be estimated. Our starting *cmr* and *cml* preparations typically induce between 250 and 500 U β -galactosidase activity in A348 (pSM30); this activity approximately corresponds to between 0.5 and $1 \mu\text{M}$ AS.

Biological activity of acetyosyringone

The primary molecular signal for the initiation in *Agrobacterium* of the events of plant cell transformation should induce in the bacterium both the entire *vir* regulon and the initial steps of T-DNA transfer. We determined that AS has these activities. Several *vir::lac* and *pin::lac* *Agrobacterium* strains were incubated in $20 \mu\text{M}$ AS (both commercial and *cmr*-purified) and assayed for β -galactosidase activity. Inductions of all the previously identified inducible *vir* loci (*B*, *C*, *D*, *E*, *G*) and *pinF* was obtained, and the levels of induction were at least 20–50% higher than those stimulated during co-cultivation with plant cell cultures or incubation with *cmr*¹⁷ (data not shown). AS also induces the production of T-DNA intermediates: when bacteria are incubated with $20 \mu\text{M}$ AS for 12–18 h, T-DNA intermediates¹³ are found at a frequency two- to fivefold greater than that obtained following 48-h co-cultivation with protoplasts¹⁷ (data not shown). We have also seen that AS induces the appearance and/or disappearance of several major proteins in *Agrobacterium* (P. Engström, P.Z. and S.E.S., in preparation). Thus, a single compound is sufficient to trigger the complete activation of *vir* and the initial events of T-DNA transfer; and AS (probably OH-AS also) is a primary signal for plant cell transformation by *Agrobacterium*.

Molecular specificity of *vir* induction

Table 1 shows the respective *vir*-inducing activities of AS (*a*) and analogues of AS (*b–g*). These results indicate that *Agrobacterium vir* expression is efficiently induced by molecules that conform to a structure best represented by AS itself, and suggest that *Agrobacterium* has evolved to recognize and respond to AS as a specific signal for the activation of plant cell transformation. For instance, AS without one methoxy group (*b*) has greatly attenuated *vir*-inducing activity, whereas AS without both methoxy groups (*d*) or its hydroxyl group (*c*) is inactive. The acetyl substituent of AS is important for activity. The formyl (*e*) and carboxylic acid (*f*) analogues of AS have attenuated activity, whereas the cinnamic acid analogue of AS, sinapinic acid (*g*), has approximately equivalent activity to AS. This latter result is interesting in that sinapinic acid is a precursor of lignin, an integral cell-wall constituent of all vascular plants (see below).

Wounding stimulates AS/OH-AS production

Although many dicotyledonous plant cells can be transformed by *Agrobacterium*, only cells that have been wounded or traumat-

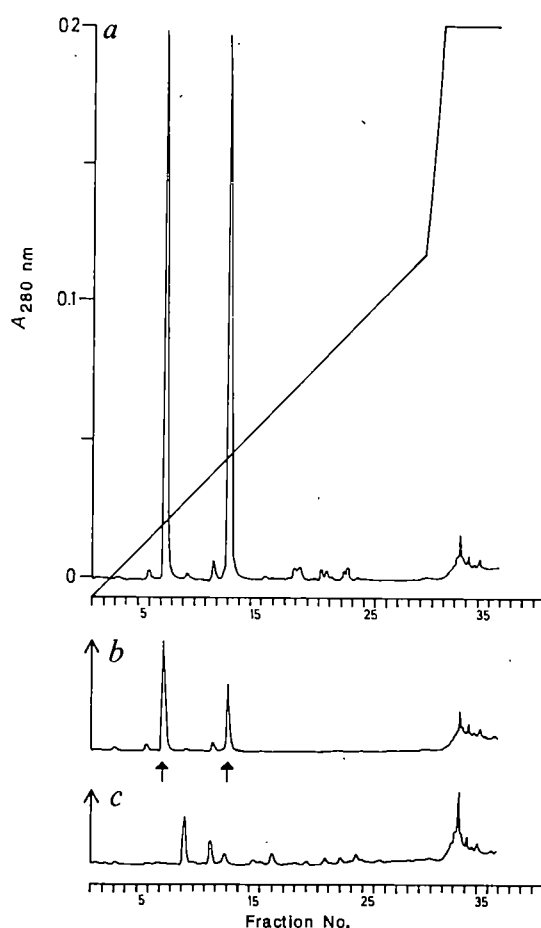


Fig. 5 Effect of wounding and inhibition of plant cell metabolism on production of AS and OH-AS by plant cells. RPC/FPLC fractionations of equivalent amounts of medium conditioned with: *a*, 1.5-cm-diameter leaf disks; *b*, intact leaves; *c*, same as *a* but with 5 p.p.m. (12.8 μM) cycloheximide (Actidion, 99%; Aldrich Chemicals) added to the medium. After a 72-h incubation, 40 ml of each respective conditioned medium was recovered, filtered through 0.22 μm nitrocellulose and 35 ml extracted with chloroform (Fig. 1), and the organic pellet was resuspended in 400 μl 10% CH_3OH , 0.1% CH_3COOH . We analysed 100 μl of the sample by RPC/FPLC using the conditions described in Fig. 1. The remaining 5 ml of conditioned medium was bioassayed for *vir*-inducing activity; the units of β -galactosidase activity induced by the conditioned media of *a*, *b* and *c* were 565, 135 and 20, respectively.

Methods. Leaves (4 g) excised from 6-week-old *N. tabacum* SR1 plants were treated as described below and incubated in 50 ml MS medium in a 150-ml Petri dish. Care was taken to use visually equivalent leaves in each experiment. *a*, 5–6 1-cm² disks were obtained per leaf; *b*, intact leaves were used; *c*, same as *a* but with 5 p.p.m. (12.8 μM) cycloheximide (Actidion, 99%; Aldrich Chemicals) added to the medium. After a 72-h incubation, 40 ml of each respective conditioned medium was recovered, filtered through 0.22 μm nitrocellulose and 35 ml extracted with chloroform (Fig. 1), and the organic pellet was resuspended in 400 μl 10% CH_3OH , 0.1% CH_3COOH . We analysed 100 μl of the sample by RPC/FPLC using the conditions described in Fig. 1. The remaining 5 ml of conditioned medium was bioassayed for *vir*-inducing activity; the units of β -galactosidase activity induced by the conditioned media of *a*, *b* and *c* were 565, 135 and 20, respectively.

ized are susceptible. Therefore, we tested whether the production of AS and OH-AS is stimulated by plant cell wounding. Figure 5 shows the RPC/FPLC profiles of the organic extracts of *cml* produced by equivalent amounts of *N. tabacum* leaf disks (wounded cells; Fig. 5a) or intact leaves (unwounded; Fig. 5b). Comparison of these profiles indicates that the 'wounded' *cml* contains >10-fold more AS and OH-AS than the 'unwounded' *cml*, demonstrating that wounding stimulates the appearance of these molecules in the cell exudate. The low levels of AS and OH-AS in the 'unwounded' leaf exudate could be caused by the cut stem surfaces of the leaves.

These results do not define which cells of the wounded tissue produce AS and OH-AS. For example, damaged or dead cells could release AS, although such cells are not good targets for

Agrobacterium infection. We tested whether active plant cell metabolism is required for the production of AS by analysing the *cml* produced by leaf disks incubated in cycloheximide. This material does not contain AS and OH-AS (Fig. 5c). These results confirm and extend our previous observation that in plant/*Agrobacterium* co-cultivations only actively growing plant cell cultures are able to stimulate efficient *vir* gene expression¹⁷. Our data concerning the production of AS and OH-AS in total suggest that these compounds are specifically synthesized and exuded by metabolically active wounded cells.

Discussion

The molecules that we have purified signal *Agrobacterium* to activate the expression of its virulence genes, setting in motion a series of molecular events ultimately resulting in the transfer of T-DNA from the bacterium to the plant cell.

Wounded cells are known to be susceptible to *Agrobacterium* infection, perhaps because the intact cell walls of undamaged cells restrict T-DNA transfer, or because T-DNA integration is dependent on host-cell replication and wounding stimulates this replication. Because *Agrobacterium* responds to AS and OH-AS to initiate plant cell transformation, these molecules potentially represent the signal that *Agrobacterium* detects and recognizes in the soil as susceptible plant cells. The presence of AS and OH-AS specifically in the exudates of wounded but actively metabolizing plant tissues supports our hypothesis.

The activation of *Agrobacterium vir* expression by plant signal molecules probably involves at least two steps: extracellular recognition and intracellular response. The first step could depend on the signal molecule acting as a chemical attractant and/or nutritive source for the bacterium. The latter must depend on the ability of the bacterium to convey the information of the signal from outside to inside the cell and to activate *vir* expression. The mechanism of these events is unknown. Induction of the pTiA6 *vir* region is attenuated in *virA* and does not occur in *virG* mutant bacteria (S.E.S., in preparation). Also, the amino-acid sequence of the *virG* gene product is highly homologous with several positive regulatory proteins of *E. coli*²¹. Because AS can cause the induction of the complete *vir* regulon, we suggest that this compound acts to activate allosterically the *virG* protein, which then activates *vir* transcription by directly interacting with *vir* gene promoter sequences. The *virA* protein potentially functions in the initial extracellular/intracellular recognition and/or intracellular transport of the signal molecule.

AS and OH-AS have not previously been identified as natural component of plant cells, suggesting that the appearance of these molecules in nature is not widespread. Thus, these molecules potentially represent to *Agrobacterium* only those cells which are its desired targets. In addition, these or related molecules might also serve to initiate other bacterial/plant interactions in the soil. The observed resistance of most monocotyledonous plants to *Agrobacterium* could result because these

plants do not produce, or only produce in low quantities, *vir* signal molecules. AS and OH-AS could be useful for obtaining *Agrobacterium* transformation of plant species previously seen to be resistant to *Agrobacterium*, and also for the analysis of the initial molecular steps of the T-DNA transfer process.

Although it is important for *Agrobacterium* to detect and respond to such molecules, it is equally important that it does not respond to closely related but functionally different molecules; we have shown that *vir* induction is most efficiently stimulated by AS and by the lignin precursor sinapinic acid. This latter compound is not present in detectable quantities in our exudate preparations; however, sinapinic acid could be present in the soil in exudates of wounded cells in the process of cell-wall rebuilding.

It is interesting to speculate on the function of AS and OH-AS in plants. These compounds are likely products of the shikimic acid biosynthetic pathway that provides the plant cell with the precursors to a broad spectrum of molecules, including the flavonoids, and lignins²²⁻²⁴. These classes of compounds are important to a plant subjected to stress or injury. For example, many flavonoid-derived phytoalexins are potent inhibitors of the growth of invading pathogens²⁵, whereas lignin, a major component of the cell wall, provides a physical barrier to invasion²⁶. Thus, AS and OH-AS could be part of the wound response²⁷ of plant cells. These compounds are potentially toxic to other soil pathogens, and *Agrobacterium* has evolved to be resistant to AS and OH-AS and to use these chemicals to recognize wounded cells. Alternatively, the compounds could be products related to lignin repair in damaged cells.

We propose that there is a continual excretion of wound-related phenolics during growth, caused by abrasion from the soil. *Agrobacterium* may be attracted to plants by recognition of these compounds. However, significant levels of *vir* induction and the events of T-DNA transfer will only occur if signal molecules are present in sufficient concentrations. As the highest concentrations are found at wound sites, *A. tumefaciens* effectively infects only these sites.

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The *Drosophila* developmental gene, *engrailed*, encodes a sequence-specific DNA binding activity

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Plasmid expression vectors carrying either the entire engrailed coding region or a subfragment including the homoeo box, produce protein fusions having sequence-specific DNA binding activity.

MUTATIONS in *Drosophila* have identified genes that control major steps in development¹⁻³. Some of these mutants, the segmentation mutants, are defective in the processes that subdivide the embryo into the segmented body plan⁴⁻⁷ while others, the homoeotic mutants, improperly specify the developmental fate of particular regions of the fly¹. Garcia-Bellido⁸ suggested that these mutations affect 'selector genes' that act, in those cells in which they are expressed, to select the developmental pathway. It was proposed that they function by controlling 'cytodifferentiation genes'⁸.

A segmentation gene

Each morphologically obvious segment is composed of cells from two distinct lineages termed anterior and posterior compartments^{9,10}. Genetic analysis suggests that the *engrailed* gene product is required to specify cells as members of posterior compartments¹¹⁻¹⁴. As anticipated by the selector gene hypothesis⁸, by 3.5 h of development *engrailed* gene product accumulates in narrow bands corresponding to the primordia of the posterior part of each segment¹⁵⁻¹⁷. Apparently, the

engrailed regulatory activity acts wherever it is expressed to direct cells along a pathway of development suited to cells of posterior compartments⁸. In *engrailed* mutants, the segmental fusions and the failure to specify cells of posterior compartments are thought to result from the absence of this regulator or from alterations in its expression.

Selector genes interact

In addition to *engrailed*, several other *Drosophila* developmental genes are expressed in spatially restricted patterns consistent with their apparent roles in directing particular portions of the developmental programme¹⁵⁻¹⁹. These studies have focused interest on two related issues. How are these genes regulated to achieve the appropriate pattern of expression, and how do they regulate subsequent development? Recent studies suggest that the selector genes interact in a complex regulatory network. Based on phenotypes of double mutant combinations, Struhl²⁰ argued that the *Ubx* gene product represses *Scr* expression in the mesothorax. Molecular studies²¹ have offered further suggestions of interactions. Regulatory interactions among six different

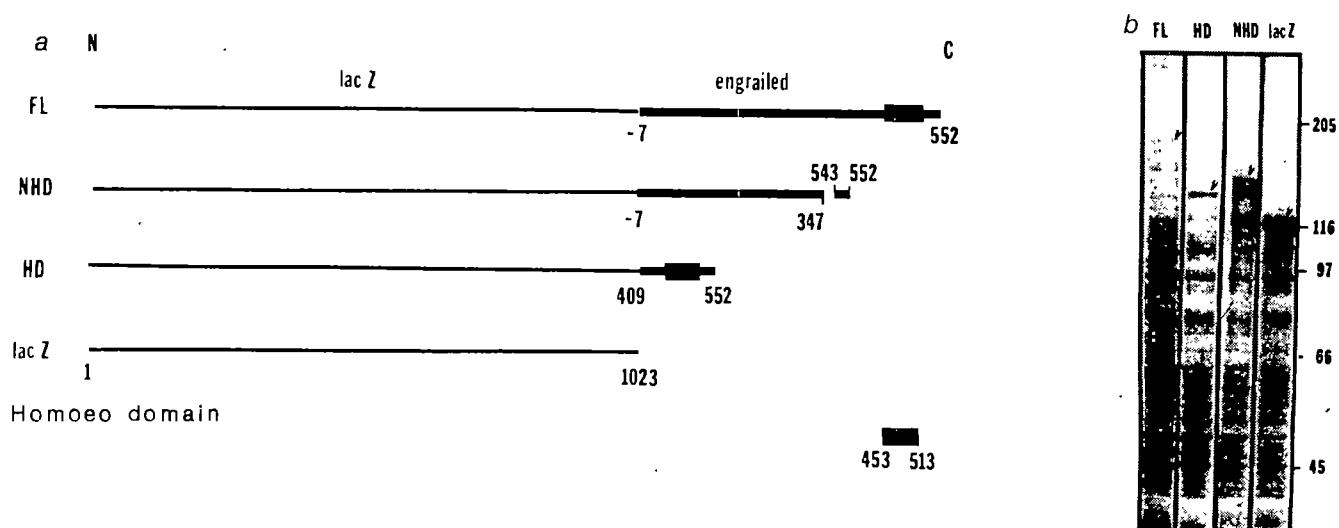


Fig. 1 Construction of *lacZ-engrailed* fusions and expression in *Escherichia coli*. *a*, Gene fusions; *b*, polyacrylamide gel electrophoresis of bacterial extracts; FL, full-length fusion; HD, homoeo domain fusion; NHD, non-homoeo domain fusion; *lacZ*, β -galactosidase.

Methods: *a*, DNA fragments derived from the *engrailed* locus^{17,35,36} were inserted in the polylinker of expression vector plasmids pUR 290, 291 or 292 (ref. 47). These different plasmids allow inserted DNA to be expressed as a C-terminal extension of β -galactosidase. In the full-length fusion protein (FL) the extension includes 7 amino acids that precede the first methionine of the *engrailed* protein as well as the entire *engrailed* protein. In the homoeo domain construct (HD), a *Bam*HI fragment was spliced out of the FL construction, keeping only the last quarter of the *engrailed* coding sequence from amino acid 409 to the end. This fusion protein contains the entire homoeo domain (amino acids 453-513). Amino acids 347-542, containing the homoeo domain, have been deleted in the non-homoeo domain protein (NHD) by removing a *Xho*I fragment from the FL construct. In the absence of insertion the *lacZ* gene is expressed as the complete β -galactosidase. *b*, Polyacrylamide gel electrophoresis of bacterial extracts. The expression of the fusion proteins from chimaeric plasmids is under the control of the *lac* promoter in a *lacI* overproducing strain DG101 (a gift from D. Gelfand, Cetus Corporation). Bacteria in exponential growth were induced with isopropyl β -thiogalactoside at an absorbance of 0.5 (at 600 nm) and collected 2 h later. Cells were resuspended in about 0.005 culture volume of 25% sucrose, 0.2 mM EDTA, 40 mM Tris-HCl pH 7.5 and 1 mM dithiothreitol (DTT). Lysis and protein solubilization were achieved by lysozyme treatment (0.4 mg ml⁻¹ for 1 h at 0 °C followed by addition of urea to 4 M and further incubation at 0 °C for 1 h). After centrifugation at 20,000 r.p.m. for 1 h the urea was removed from the supernatant by dialysis first against 10 mM Tris-HCl pH 7.5, 25 mM NaCl, 1 mM EDTA, 0.1% Triton X-100, 1 mM DTT, 10% glycerol, 1 mM phenylmethylsulphonyl fluoride, and 0.1 mM benzamidinium containing 2 M urea and subsequently against the same buffer without urea. Glycerol was added to the extracts to a final concentration of 50% and they were stored at -20 °C. When analysed by SDS-gel electrophoresis the fusion proteins are seen as abundant high relative molecular mass proteins (arrowheads). The larger fusion protein is present in lower amounts and we detect numerous minor bands that are presumed to result from degradation of the fusion proteins.

homoeotic loci appear to coordinate their spatial patterns of expression (ref. 22 and C. Wedeen and M. Levine, personal communication).

Recent molecular analyses suggest that the segmentation genes interact to control the expression of one another. Immunofluorescent staining revealed that *engrailed* protein appears first in alternate segments and only later in every segment¹⁷. This led to the suggestion that *engrailed* expression is regulated by the products of another class of segmentation genes, the pair-rule genes^{17,23}, which are expressed in alternate segments^{19,24}. Alterations in the pattern of *engrailed* expression in pair-rule mutants have confirmed this prediction (S. DiNardo and P.H.O'F., unpublished observations; K. Howard and P. Ingham, personal communication). Similar analyses of *fushi tarazu* expression suggest that its expression is also influenced by several other segmentation genes (S. Carroll and M. Scott, personal communication).

These observations suggest an extensive network of regulatory interactions among the *Drosophila* developmental genes, and imply that selector genes are themselves targets for the regulators they encode^{17,23}.

The homoeo box

The products of a number of the developmental genes include a conserved protein domain of 60 amino acids, the homoeo domain. Related sequences have been identified in species from human to annelids²⁵⁻³⁰. This remarkable conservation suggests that the homoeo domain has common physical interactions in all these organisms. A portion of this domain is found in two yeast proteins, $\alpha 1$ and $\alpha 2$, that determine the cell fate (mating type) via transcriptional regulation^{31,32}. Because of this homology it has been proposed that the developmental genes of *Drosophila* might function similarly, by interacting with DNA^{27,33}. Further, it has been noted that sequences within the homoeo domains are compatible with a protein structural motif that characterizes bacterial sequence-specific DNA binding proteins^{27,34}.

Here we address three issues. Does a homoeo-domain-containing protein bind to DNA? Is the binding specific? Is the homoeo domain responsible for binding?

Construction of *engrailed* fusion proteins

To study how the *engrailed* protein product might function as a regulator, we constructed bacterial expression vectors that encoded the *engrailed* protein as carboxy-terminal extensions of β -galactosidase. In order to test for possible autonomous functions of the homoeo domain, we constructed three fusion proteins (Fig. 1). The full-length fusion contained the sequence encoding the entire 552 amino acids of the *engrailed* protein. This *engrailed* protein sequence has been derived from an open reading frame in the *engrailed* cDNA sequence³⁵. The pattern of evolutionary sequence conservation of this open reading frame suggests that it encodes protein (J. Kassis, D. K. Wright, and P.H.O'F., unpublished observations). We think that this predicted protein is made *in vivo* because two antisera directed against different domains of this predicted sequence detect expression of these domains in the posterior compartments of segments¹⁷. The 'homoeo domain fusion' includes only the terminal quarter of the protein coding sequence, encompassing the 60-amino-acid homoeo domain and an additional 44 amino acids on the N-terminal side plus 39 amino acids on the C-terminal side. The 'non-homoeo domain fusion' is deleted for a 196-amino-acid region and lacks the homoeo domain (Fig. 1).

Nonspecific DNA binding

We first tested whether the fusion proteins would bind DNA nonspecifically. We mixed the fusion proteins with labelled restriction fragments of DNA and then, using an antibody directed against bacterial β -galactosidase and fixed *Staphylococcus aureus* as an immunoadsorbent, we precipitated the fusion protein along with bound DNA fragments^{31,36}. The bound DNA fragments were then separated by electrophoresis and detected

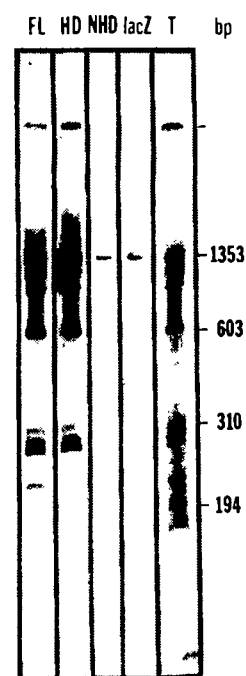


Fig. 2 Nonspecific binding of the fusion proteins to DNA. Bacteriophage Φ X174 DNA was cleaved by *Hae*III and end-labelled using T4 polymerase⁴⁸. The labelled DNA (about 30 ng) was incubated for 30 min at 0 °C in 25 μ l of binding buffer (BB) (50 mM NaCl, 20 mM Tris pH 7.6, 0.25 mM EDTA, 1 mM DTT, 10% glycerol) in the presence of a bacterial extract containing the fusion protein (about 10 μ g of total protein extract, see Fig. 1 legend). The complexes formed between DNA and the fusion proteins were then immunoprecipitated by 30 min further incubation at 0 °C with an anti- β -galactosidase monoclonal antibody (provided by Tom Ma-on and Judy Partaledis, courtesy of Mike Hall) preadsorbed on cross-linked *Staphylococcus* (Pansorbin, Calbiochem). The pellet was washed twice in BB, phenol extracted and the DNA ethanol precipitated before polyacrylamide gel electrophoresis and autoradiography. The autoradiogram shows the results of precipitations performed in the presence of extracts containing the full-length fusion (FL), the homoeo domain fusion (HD), the non-homoeo domain fusion (NHD) or β -galactosidase (*lacZ*). Lane T shows the labelled digest before immunoprecipitation, representing 25% of the counts added to the incubation mixture in the immunoprecipitation experiments.

by autoradiography. At low salt concentration (50 mM NaCl) and in the absence of carrier DNA, the full-length fusion protein and the homoeo domain fusion protein bound all of the *Hae*III restriction fragments of bacteriophage Φ X174 DNA, whereas neither β -galactosidase alone, nor the non-homoeo domain fusion protein bound significant amounts of DNA (Fig. 2). Though these observations are consistent with the hypothesis that the homoeo domain would function in DNA binding, the results could potentially be due to simple ionic interactions. Sequence-specific binding would suggest that the fusion protein has a DNA binding domain.

Sequence-specific DNA binding

Since we had no knowledge of what the target sequences for binding of *engrailed* protein might be, we sought a generalized approach to detect sequence specificity. Sequence-specific DNA binding proteins recognize degenerate versions of a consensus binding site. Sufficiently complex DNA should contain, by chance, sequences recognized by the protein. For example, Ross and Landy³⁷ identified the sequence of several binding sites for λ integrase (Int protein) in pBR322 DNA.

To pursue this approach, we digested λ -phage DNA to produce more than 100 fragments and labelled their 3' ends. This DNA was used in the assay described above, except that we added increasing amounts of salt or carrier DNA so that only fragments that bound to the fusion proteins with higher affinities would appear in the precipitate. Figure 3 shows that at low concentrations of carrier DNA all the λ DNA fragments are bound nonselectively (lanes 2, 5), but as the stringency of the

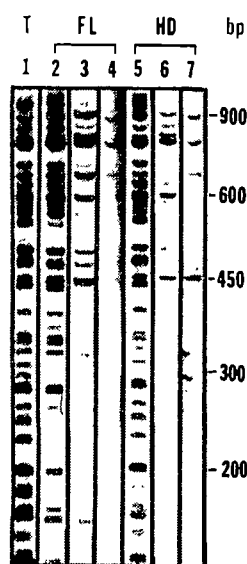


Fig. 3 Sequence-specific DNA interaction of the fusion protein with bacteriophage λ DNA fragments. Bacteriophage λ DNA was restricted with *Sau*3A and labelled using T4 polymerase⁴⁸. Binding assays were performed as described in Fig. 2 legend with the addition of carrier DNA as indicated. Fragments were separated on a 5% polyacrylamide gel. Lane 1 shows the total digest. The amount of DNA loaded in lane 1 is one-quarter of the input amount used in the binding assays (10 ng; $0.4 \mu\text{g ml}^{-1}$). Lanes 2, 3 and 4 show immunoprecipitates obtained following addition of the full-length fusion extract in the presence of 0, 4 and $40 \mu\text{g ml}^{-1}$ of carrier DNA (fragmented calf thymus DNA), respectively. Lanes 5, 6 and 7 show the results of a similar experiment using the homoeo domain fusion extract. Lanes 4 and 7 were exposed four times longer because of the reduced recovery of bands at high levels of carrier.

binding conditions is increased the binding becomes selective. For example, at high concentration of carrier DNA (Fig. 3, lanes 4, 7), only 4 fragments out of 115 DNA fragments are retained. These experiments demonstrate the specificity of *engrailed* fusion protein binding to DNA.

Whether the homoeo domain fusion or the full-length fusion is used in binding assays, the same fragments are recovered in immunoprecipitates at comparable efficiencies (Fig. 3). Thus, the two fusions bind with the same specificity and generally exhibit similar relative affinities for these fragments. The 143 amino acids of the *engrailed* sequence present in the homoeo domain fusion must include a domain competent in specific binding. At least under the conditions of our assay, the additional 409 amino acids of the full-length fusion protein make little or no contribution to the specificity of binding.

To estimate the minimal binding constant of the binding interaction, we assume that all of the fusion protein is active^{31,36}. When our binding assay contains less than 10^{-8} M fusion protein and less than 10^{-11} M DNA fragments, recovery of specific DNA fragments in the immunoprecipitate exceeds 50% (for example Fig. 4A, *engrailed* fragments f and k). Accordingly, the binding constant must exceed $5 \times 10^7 \text{ mol}^{-1}$ (at 170 mM NaCl). Furthermore, preliminary evidence indicating that only a fraction of the fusion protein is active (J.T., unpublished observations) suggests that the binding constant must be higher, and may well be comparable to the binding constants of other sequence-specific DNA binding proteins³⁴.

The binding behaviour of the fusion protein described here may not accurately reproduce the behaviour and specificities of the natural *engrailed* protein. The binding specificity might be influenced by interactions that the fusion protein cannot reproduce or by modifications that would be missing in a protein produced in *Escherichia coli*. Furthermore, our simple *in vitro* binding assay may lack accessory factors that influence *in vivo* binding of the *engrailed* protein to DNA. Nonetheless, because other work using fusion proteins or proteolytic fragments^{31,34} suggests that DNA binding domains can function relatively autonomously, we believe that the results reported here are likely to reflect at least a subset of the activities of the normal *engrailed* protein.

Specific binding to *Drosophila* DNA

If *engrailed* and other selector genes act as pleiotropic regulators of transcription, we might expect their protein products to interact with DNA near the promoters of a number of target genes. Can we identify any plausible candidates for target genes? There is considerable evidence that selector genes regulate each other's expression (summarized above). Thus, we envision that the developmental genes will include regulatory sites that are targets for interaction with the products of other developmental genes. Because of the high degree of relatedness of the developmental genes, the various target sites might also be homologous. Because it is a member of this group of related proteins, perhaps the *engrailed* fusion protein will exhibit site-specific interaction with all or a subset of the related regulatory sites.

Following this line of logic, we decided to look for *engrailed* fusion protein binding adjacent to cloned selector genes. Because a detailed analysis would require DNA sequence information, we chose to examine the *engrailed* locus itself, for which we have 1.2 kilobases (kb) of upstream sequence (unpublished data), and the *fushi tarazu* locus that had been sequenced by Laughon and Scott²⁷.

We looked for *engrailed* fusion protein binding to a 4.9-kb *Eco*RI fragment that includes 2.6 kb of *engrailed* coding sequence and 2.3 kb of upstream sequences³⁸ and to a 3.2-kb fragment that includes the *fushi tarazu* coding sequence and flanking sequences⁴⁹. Figure 4A shows that both the cloned *engrailed* sequences and cloned *fushi tarazu* sequences contain fragments that bind to the *engrailed* fusion protein under stringent assay conditions. In fact, a number of binding fragments are detected (Fig. 4A, C). The positions of binding fragments are indicated in Fig. 4B.

We purified the subfragments indicated by the hatched lines in Fig. 4B and used these to map more precisely the binding interactions upstream of the *engrailed* and *fushi tarazu* coding regions. Secondary digests of these fragments were tested for interactions with the *engrailed* fusion protein (Fig. 5). These analyses localized three binding sites within the 900-base-pair (bp) region 5' to the *engrailed* cDNA. The higher resolution and sensitivity of these experiments showed that the binding fragment k (Fig. 4A, B) actually contained two binding sites (sites a and b in Fig. 5) and that fragment d, though not detected as a binding fragment in experiments using the whole plasmid, contains a weak binding site (site c in Fig. 5). The analysis of the *fushi tarazu* subfragment did not reveal any new binding sites but did contribute to more accurate localization of the upstream site (Fig. 5). At present the accuracy of localization of the sites does not allow us to identify a consensus binding site unambiguously.

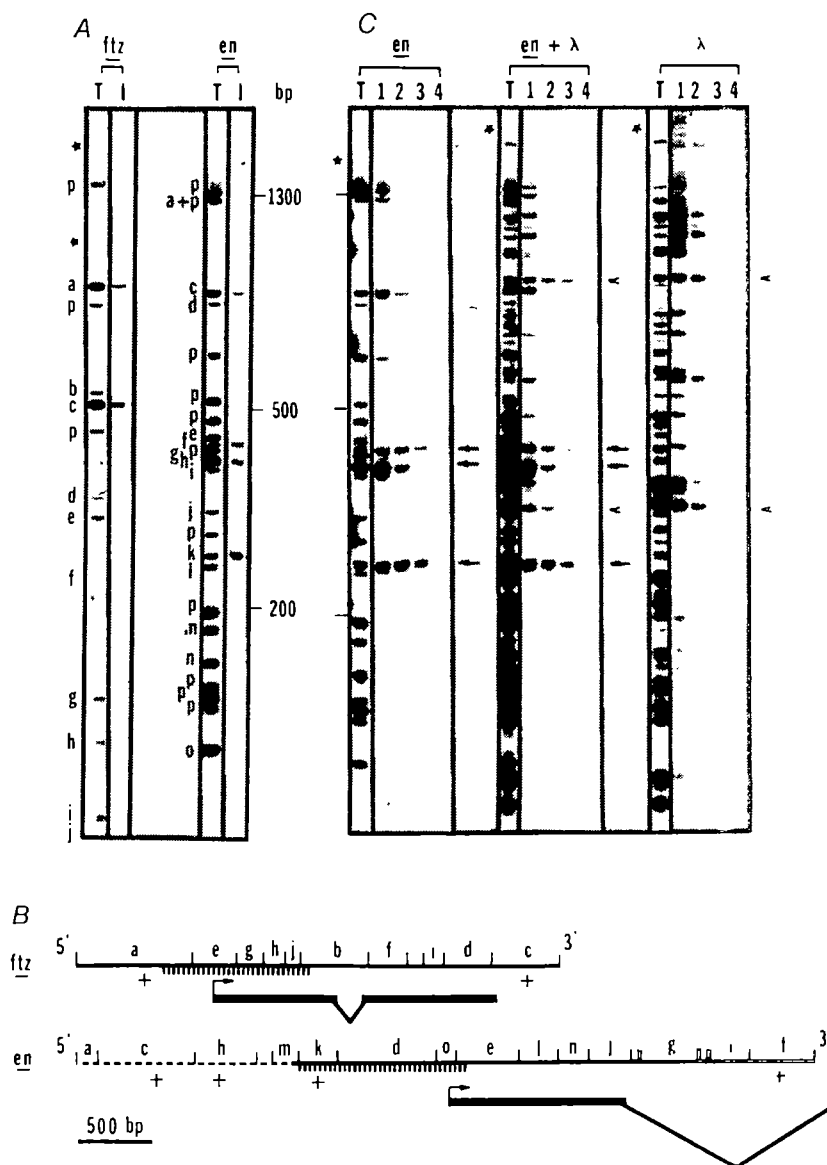
Binding sites

Without a functional assay we cannot directly assess the importance of the binding sites detected in cloned *Drosophila* sequences. However, we can test whether affinities, frequency of occurrence, clustering and location of binding sites differ from fortuitous sites.

If the frequency of fortuitous sites were extremely low (less than 1 per 1,000 kb), the presence of a cluster of binding sites within a few kilobases of *engrailed* DNA would be highly significant. This is not the case. Although the frequency of binding sites on a 4.9-kb fragment of *engrailed* DNA is higher than the density of λ DNA, the difference was not very large—about 10-fold (Fig. 4C).

Fortuitous binding sites should have a wide range of affinities depending on their similarity to an optimal site. Higher-affinity fortuitous sites should be less frequent (chance might produce an optimal binding site but should do so less frequently than imprecise approximations of this sequence). We tested the relative affinity of the *engrailed* fusion protein for various binding sites. We used conditions where a number of labelled restriction fragments are bound selectively. Addition of cold competitor DNA displaced bacteriophage λ DNA fragments with different

Fig. 4 Sequence-specific interaction of the homoeo domain fusion protein with restriction fragments of cloned *fushi tarazu* and *engrailed* sequences. The *fushi tarazu* clone (p6-3, derived from clone pDm439, a gift from Matt Scott)⁴⁹ contains 900 bp of upstream sequence, the entire coding sequence and 800 bp of downstream sequence. The *engrailed* clone (p615) contains 2.3 kb of upstream sequence, the complete first exon and most of the first intron^{35,38} (panel B). Restriction fragments of p6-3, p615 or λ DNA were end-labelled and tested for fusion protein binding as described in the legend for Fig. 2, except that a higher salt concentration (170 mM NaCl) was used to diminish nonspecific interactions. Fragments were separated on a 5% acrylamide gel. In the example shown in A, the *fushi tarazu* plasmid (ftz) DNA was digested with *RsaI* and the *engrailed* plasmid (en) was digested with *HinfI* and *ClaI*. Lanes marked T show the total fragment pattern and lanes marked I show the fragments recovered in the immunoprecipitate. For both plasmids the amount of DNA loaded in T is one-quarter of the amount subjected to immunoprecipitation and displayed in I. Bands derived totally from plasmid sequences are identified with a 'p'. Bands derived at least in part from insert sequences are designated as a to j (ftz) or a to o (en) and their positions within the cloned sequence are indicated in B. Similar experiments using *AluI*, *HaeIII*, *BstNI*, *DdeI* or *HpaII* to digest these plasmids gave comparable results (not shown). B shows a map of the inserts in p6-3 (ftz) and p615 (en). The orientation of the coding region within the insert is indicated. Except for the dashed portion of the *engrailed* insert, the sequence is known (refs 27, 35, and J. Kassiss, D. K. Wright and C.D., unpublished). The positions of restriction enzyme sites (*RsaI* sites in ftz and *HinfI* and *ClaI* sites in en) are indicated and all fragments detectable in the separation shown in panel A are lettered. Those fragments detected as bound by the homoeo domain fusion protein are indicated as +. Because they were not detected, a few small restriction fragments (unlettered) could not be scored in the binding experiment (A) but analysis of these DNAs cleaved with other restriction enzymes failed to detect additional binding sites. Exons of the transcribed region are indicated with a bold line and introns with sloping lines. The hatched region indicates the position of a subfragment that was purified and used for additional binding studies (Fig. 5). Note that the region responsible for binding of ftz fragment a, was further localized by analysis of *AluI*, *HaeIII*, *BstNI*, *DdeI*, *HpaII* and *HinfI* digests that defined only one site; this site corresponds to that mapped in more detail by analysis of the purified subfragment (Fig. 5). C Compares the efficiency of homoeo domain fusion protein binding to *engrailed* or λ sites. The binding assays were performed as described in A except that increasing amounts of unlabelled DNA (*HinfI*-restricted p615) was added as competitor. Lanes T represent 10% of the counts of the total digest submitted to immunoprecipitation in lanes 1, 2, 3 and 4. In lane 1, no unlabelled DNA was added while in lanes 2, 3 and 4, 30 ng, 250 ng and 4 μ g, respectively, were added. en lanes: plasmid p615 was restricted with *ClaI* and *HinfI*, labelled and submitted to immunoprecipitation. Lanes 1, 2, 3 and 4 represent immunoprecipitated DNA from ~4 ng of labelled DNA (~1 fmol). Obviously the four bands retained in A behave differently when unlabelled competitor DNA is added. Three bands (arrows) corresponding to fragments f, k and h in A, are detected in the immunoprecipitate in the presence of 1,000-fold excess of unlabelled DNA. λ lanes: λ DNA was restricted with *ClaI* and *HinfI*, labelled and processed under the same conditions as for en DNA. An equimolar amount of DNA (1 fmol) was used in this experiment (20 ng). Two bands were retained at the highest stringency (arrowheads). en + λ lanes: T is an equimolar mixture of en and λ DNA restricted with *ClaI* and *HinfI*. The bands that are retained are the same as those seen in the experiments testing en and λ DNAs individually. Five bands are retained in the presence of the highest concentration of competitor (arrows and arrowheads).



efficiencies (Fig. 4C). Thus, as expected, the binding sites in λ DNA have a range of affinities and there are few high-affinity sites. Assuming that the binding sites in λ DNA occur by chance, the specific binding of 14 restriction fragments at intermediate stringencies suggests that the sequence recognized by the *engrailed* fusion protein is relatively short (about 6 bp) or substantially degenerate.

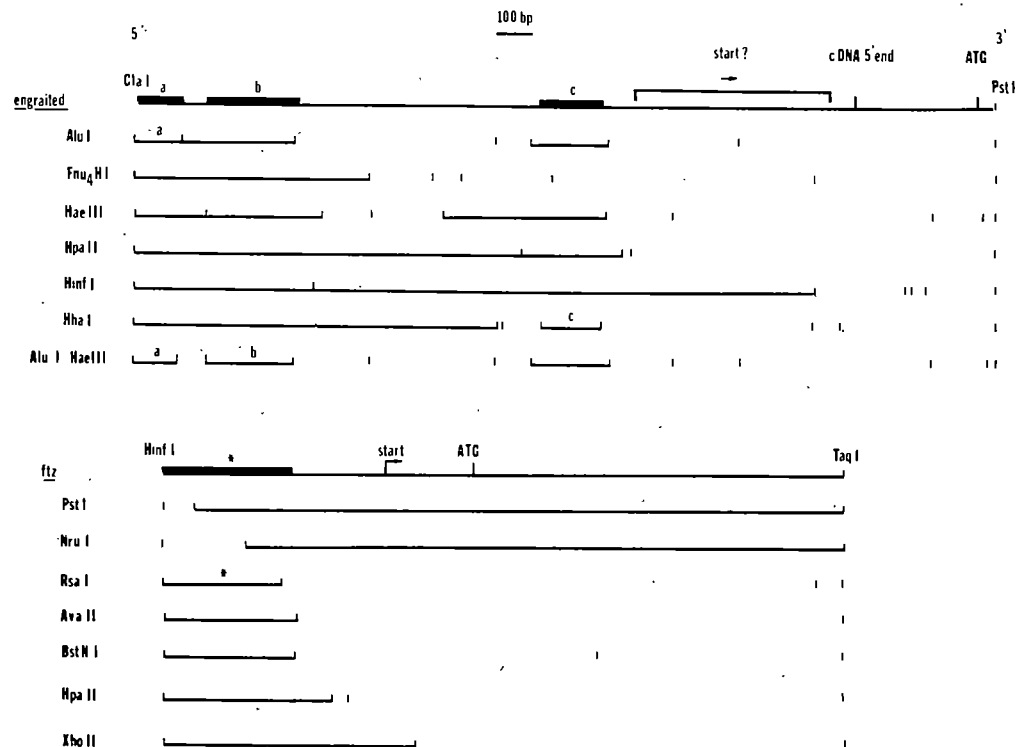
For some sequence-specific DNA binding proteins (such as *lac* repressor), fortuitous occurrence of high-affinity binding sites is extremely unlikely. For these a site of functional interaction (the *lac* operator³⁹) has a distinctively high affinity. For other sequence-specific interactions (for example λ integrase^{37,40}) the affinities of fortuitous and functional sites overlap. Depending on the characteristics of the *engrailed* fusion protein, functionally relevant sites might have distinctively high affinities. We therefore examined the relative binding affinity of *engrailed* and bacteriophage λ DNA fragments. We observe differing affinities for the *engrailed* fusion protein interaction with various sites on *engrailed* DNA (Fig. 4C). Using our present assay, the ranges of affinities seen for λ and *engrailed* sites overlap (Fig. 4C). Three *engrailed* fragments bind with

particularly high affinity (arrows) compared with two λ fragments (arrowheads).

The binding data provide no support for suggestions that the binding sites in *Drosophila* DNA are functional. It should, however, be made clear that the opposing conclusion also cannot be reached from these data; that is, the binding sites in *engrailed* DNA cannot be dismissed as nonfunctional because they have properties similar to fortuitous binding sites. Thus, the issue of function remains open.

Although the functional importance of the binding sites still requires experimental test, we propose that the binding sites we have detected in *Drosophila* DNA function *in vivo* as targets for interaction with either the *engrailed* protein or closely related gene products (that is, other selector genes). We make this suggestion on the basis of the location, clustering and conservation of the sites. The positions of binding sites in relation to the *fushi tarazu* and the *engrailed* coding regions are reminiscent of the positions of enhancer elements in other systems⁴¹⁻⁴³. The clustering of binding sites is unlikely to be coincidental. Such clustering of binding sites for regulatory proteins is fairly common (for example, refs 44, 45). Finally, if functional, the binding

Fig. 5 Localization of binding sites in the 5' regions of *engrailed* and *fushi tarazu*. In digests of whole plasmid DNA we identified fragments that were bound by the homoeo domain fusion protein (Fig. 4). To localize more precisely the binding sites immediately upstream of the *engrailed* and *fushi tarazu* coding regions, we purified a 1,180-bp *Clal*-*PstI* fragment from the *engrailed* clone and a 939-bp *HinfI*-*TaqI* fragment from the *fushi tarazu* clone (Fig. 4B). These purified fragments were digested with additional enzymes as indicated and the binding of subfragments assayed as before (Fig. 4). In each of the restriction digests shown here the binding fragments are indicated as solid lines. Although the overlap of binding sites: a *Clal*-*AluI* fragment (a, the binding regions, this could be misleading if the binding sites were complex. Therefore, in the summary diagrams above the restriction patterns, we have indicated the precision of the localization of the binding sites based on the minimal fragment showing binding (bold line). For *engrailed*, three fragments were identified as containing binding sites: a, *Clal*-*AluI* fragment (a, 67 bp), a *HaeIII*-*AluI* fragment (b, 100 bp) and a *HhaI* fragment (c, 88 bp). For *ftz* a single site was localized to a 165-bp *HinfI*-*RsaI* fragment(*).



sites will be conserved in evolution. In the absence of a functional test, we believe that the best way to distinguish fortuitous and functional binding sites is to see whether protein binding occurs at analogous positions in distantly related genomes. We have cloned the *engrailed* gene of a distantly related *Drosophila* species, *D. virilis*⁴⁶. A preliminary analysis indicates that the fusion protein binds to fragments upstream of the *D. virilis* *engrailed* gene (D. Wright, unpublished data).

Binding specificity

Together with previous arguments²⁷, our results predict that the homoeo domain imparts a sequence-specific DNA binding activity to the protein. Accordingly, other homoeo domain-containing proteins should also bind DNA in a sequence-specific manner and such proteins having closely homologous homoeo domains should have similar sequence specificity. We presently recognize two classes of homoeo domain sequences. Class I is comprised of seven genes which have highly homologous homoeo domains and are located in two clusters of developmental genes (the bithorax complex and the *Antennapedia* complex)^{22,25}. Class II is comprised of the *engrailed* homoeo domain and the highly homologous homoeo domain of the *engrailed* related gene³⁵. The homoeo domains of different classes have lower homology (Fig. 6). As noted previously, the regions of sequence identity suggest that class I homoeo domains might specify binding to the same sequence²⁷. The differences between class I and class II homoeo domains might include an alteration of the sequence specificity.

Evolution of proteins

Duplication and divergence of a primordial gene encoding a DNA binding protein might lead to a family of interacting regulators. If the primordial protein included sequences for dimerization and for DNA binding, newly duplicated coding regions would have common binding specificities and could form heterotypic dimers. The interactions between products of duplicated genes would persist if the dimerization function and DNA binding function were conserved. We suggest that continued duplication and divergence can result in a family of DNA binding proteins that interact physically by forming heterotypic associations and interact functionally by competition for binding

to related DNA binding sites. Such interactions would link the various genes in a regulatory network. The evolution of the members of the family would be coupled because of the importance of maintaining the interactions among the members of this regulatory network. Since coordinate change of many genes is an extraordinarily unlikely event, such a network of interaction

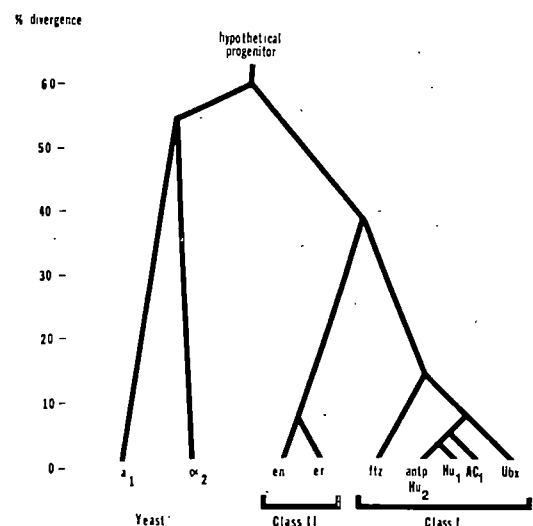


Fig. 6 Family tree of relatedness of homoeo domains. Pairwise comparisons of the protein sequences of the homoeo domains encoded by *Drosophila* genes (*Antp*, *Ubx*, *ftz*, *en* and *er*), yeast genes (*a1* and *a2*) and sequences isolated by homology from humans (*Hu1* and *Hu2*) and frogs (*AC1*) were used to score divergence. The comparison was confined to residues 29-58 because this region is well conserved among all of these sequences. To produce homology scores we gave one point for amino-acid identity and half a point for similarity. The data are assembled into a family tree by indicating the divergence of each sequence that approximates all the pairwise comparisons. The sequence data are taken from Levine *et al.*²⁸ (*Hu1* and *Hu2*), Scott and Weiner²⁶ (*Ubx* and *ftz*), McGinnis *et al.*²⁵ (*Antp*), Poole *et al.*³⁵ (*en* and *er*), Carrasco *et al.*³⁰ (*AC1*), and Tatchell *et al.*³² (*a2* and *a1*). Note that the discrepancies in the published sequence^{25,26} for *Ubx* are significant in comparison to the divergence among class I homoeo domains.

may contribute to the extraordinary conservation of homoeo domain sequences. It should be possible to test the predictions of this rationale using approaches similar to those used here to show that the *engrailed* gene encodes a sequence-specific DNA binding activity.

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Rusconi, Elizabeth Sher and Deann Wright. We thank Steve Poole for the gift of *en* cDNA and sequence information before publication, Matt Scott for the *fushi tarazu* clone and for encouragement, and Sandy Johnson and Keith Yamamoto for their comments on the manuscript. This work was funded by NSF grant PCM-8418263 and by NIH grant GM 31286. C.D. was supported by a Fogarty fellowship and by ARC, and J.T. by an NIH training grant.

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Primary structure and expression of a functional human glucocorticoid receptor cDNA

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Identification of complementary DNAs encoding the human glucocorticoid receptor predicts two protein forms, of 777 (α) and 742 (β) amino acids, which differ at their carboxy termini. The proteins contain a cysteine/lysine/arginine-rich region which may define the DNA-binding domain. Pure radiolabelled glucocorticoid receptor, synthesized in vitro, is immuno-reactive and possesses intrinsic steroid-binding activity characteristic of the native glucocorticoid receptor.

TRANSCRIPTIONAL regulation of development and homeostasis is controlled in complex eukaryotes by a wide variety of regulatory substances, including steroid hormones. The latter exert potent effects on development and differentiation in phylogenetically diverse organisms and their actions are mediated as a consequence of their interactions with specific, high-affinity binding proteins referred to as receptors¹⁻⁶. Many of the primary effects of steroid hormones involve increased transcription of a subset of genes in specific cell types^{7,8}. The structural characterization of the steroid receptor protein and the definition of the molecular actions of the steroid hormone/receptor complex would help to define the biochemical events which modulate gene transcription. A series of receptor proteins, each specific for one of several known steroid hormones, are distributed in

a tissue-specific fashion, although many cell types simultaneously express receptors for several steroid hormones^{9,10}. It is postulated that steroids enter cells by facilitated diffusion and bind to the specific receptor protein, initiating an allosteric alteration of the complex. As a result of this transformation, the steroid hormone/receptor complex appears capable of binding high-affinity sites on chromatin and modulating transcription of specific genes by a mechanistically unknown process^{11,12}.

The glucocorticoid receptor is widely distributed and expressed in many cultured cell lines, and the control of gene expression by glucocorticoids, therefore, has been widely studied as a model for transcriptional regulation. A number of glucocorticoid-responsive transcriptional units, including mouse mammary tumour virus (MMTV)^{13,14}, mouse and human metal-

Fig. 1 Human glucocorticoid receptor cDNA sequencing strategy and schematic representation of cDNA clones. **a**, The composite cDNA for the α glucocorticoid receptor is represented at the top, with noncoding (lines) and coding (stippled portion) sequences indicated. Common 6-nucleotide restriction enzyme sites are shown. Overlapping cDNA inserts used to determine the sequence are shown: arrows beneath the regions sequenced show the direction and extent of sequencing. The dashed line at the 3' end of OB10 indicates divergent sequence. Numbers refer to nucleotide positions in OB10 relative to the 5'-most transcribed sequence. **b**, cDNAs encoding the α and β forms of the receptor (OB7 and OB10, respectively). The 5' end of OB7 (broken lines) is contributed by the OB10 clone. Protein-coding information is represented by wide bars; untranslated sequences are indicated by thin bars. Nucleotides and amino acids are numbered above and below the coding sequence, respectively. Common DNA sequences extend to nucleotide 2,313 (amino-acid residue 727), at which point the α - and β -receptor forms diverge, with the α cDNAs (OB12, OB7) continuing in an open reading frame for 150 nucleotides (50 amino acids) and the β cDNA (OB10) continuing for 45 nucleotides (15 amino acids; see Fig. 3). Hexanucleotide signals (AATAAA) just upstream of the poly(A) in the clones are indicated, with the first hexanucleotide in OB7 serving as poly(A) in OB12.

Methods. The inserts hGR1.2, hGR2.9 and hGR5.16 were isolated from a λ gt11 IM-9 lymphoid cell cDNA library as described previously⁴². Two clones were isolated from cDNA libraries constructed by H. Okayama in pcD (ref. 44) using poly(A)⁺ mRNA from GM637 human fibroblasts (OB7) and primary human fibroblasts (OB10). Screening was performed with the hGR1.2 cDNA, radiolabelled by nick-translation with ³²P-dCTP. Sequences were determined by the chemical cleavage method of Maxam and Gilbert⁴⁵.

lothionein^{15,16}, rat α_2 -globulin¹⁷ and rat and human growth hormone¹⁸⁻²⁰ genes have been identified. DNA sequences mediating transcriptional stimulation of several of these genes have been localized. For MMTV, these sequences are discrete genomic regions upstream of the transcriptional start site which appear to exert their actions independently of orientation and position^{21,22}. The steroid/receptor complex appears to bind to these regulatory sequences and purified receptor has been used to define the specific binding sites²³⁻²⁶. Based on the footprinting analyses of several responsive genes, a consensus DNA binding sequence sharing the core sequence 5' TGT/CTCT 3' has been proposed²⁷.

The ability of the glucocorticoid-responsive element (GRE) to alter its position and orientation yet still maintain promoter inducibility suggests that it resembles the class of *cis*-acting regulatory sequences termed enhancers²¹. First discovered in viruses and subsequently in cellular genes, these sequences are necessary for efficient transcription *in vivo*²⁸⁻³¹. It has been suggested that enhancers are recognized by *trans*-acting factors that mediate regulatory effects by tissue-specific transcriptional control. Although the enhancer factors have not been well characterized, the glucocorticoid receptor may serve as a paradigm for these putative gene activator proteins.

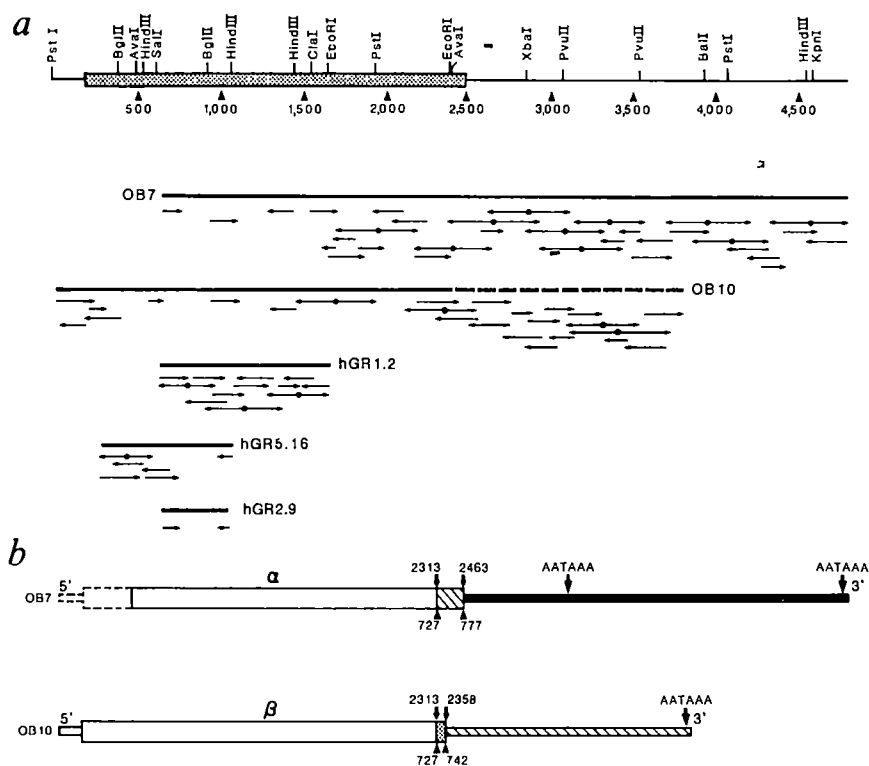
The availability of radiolabelled high-affinity glucocorticoid analogues such as dexamethasone and triamcinolone acetone has led to the development of purification strategies resulting in the isolation of nearly pure rat and human receptors^{32,33}. Although the receptor migrates as a dimer in sucrose gradients, analysis on denaturing SDS-polyacrylamide gels detects a single polypeptide of relative molecular mass (M_r) ~94,000 (94K)^{34,35}. The native polypeptide contains intrinsic specificity for steroid binding and DNA sequence recognition. By using as probes monoclonal and polyclonal antibodies raised against the purified rat and human receptors³⁶⁻³⁸, it has been possible to identify a major immunogenic region in the receptor residing on a portion of the molecule that is distinct from the steroid- and DNA-binding regions³⁹⁻⁴¹. To gain further information about the structure of this molecule and to begin an analysis of the

molecular mechanisms by which it regulates gene transcription, we set out to clone receptor cDNA sequences. By using receptor-specific antibodies as probes, we and others have isolated clones containing human or rat glucocorticoid receptor cDNA inserts^{42,43}.

Here we report the complete amino-acid sequence of the human glucocorticoid receptor (hGR), deduced from human lymphoid and fibroblast cDNA clones. The sequence reveals various structural features of the receptor, including the major immunogenic domain and a cysteine/arginine/lysine-rich region which may constitute a portion of the DNA-binding domain. We describe the use of the SP6 transcription vector system to generate analytical amounts of full-length protein, and demonstrate that the cell-free translated protein is both immunoreactive and possesses steroid-binding properties characteristic of the native glucocorticoid receptor. An accompanying paper describes the homology of the hGR sequence to that of the oncogene *v-erb-A*⁶⁴.

Glucocorticoid receptor cDNA

A library of cDNA clones was constructed in the phage expression vector λ gt11 using poly(A)⁺ RNA from the human lymphoid cell line IM-9 as template, as described previously⁴². This library was initially screened with a rabbit polyclonal antiserum to the purified glucocorticoid receptor, resulting in the isolation of several immunopositive candidate clones from ~2.5 × 10⁵ plaques. The β -galactosidase fusion proteins generated from these clones were used to affinity-purify receptor epitope-specific antibody, which was subsequently recovered and identified by binding to protein blots of cellular extracts. Three clones containing inserts expressing antigenic determinants of the human glucocorticoid receptor were isolated. The inserts of these clones, although of different sizes, cross-hybridized, indicating that they contained a common sequence which presumably delimits the major immunogenic domain of the receptor. Together, these clones spanned 1.4 kilobase pairs (kbp) but were clearly not long enough to code for the entire



[illegible]

Fig. 2 cDNA and predicted protein sequence of human glucocorticoid receptor. The complete α coding sequence and OB7 3'-untranslated region are shown, with the deduced amino acids given above the long open reading frame. An upstream in-frame stop codon at nucleotides 121-123 and putative additional polyadenylation signals in OB7 are underlined.

receptor, which was estimated to require ~2,500 nucleotides to encode a polypeptide of M_r 94K.

To isolate additional cDNA clones we again screened the original library and also examined a second library (given by H. Okayama) prepared with poly(A)⁺ RNA from human fibro-

lasts in the vector described by Okayama and Berg⁴⁴. Using one of the immunopositive cDNA inserts (hGR1.2) as probe, 12 clones were isolated that, together, covered more than 4.0 kbp. The nucleotide sequences of these clones were determined by the procedure of Maxam and Gilbert⁴⁵ according to the strategy

Fig. 4 Immunoblot comparison of hGR translated *in vitro* with *in vivo* hGR from cell extracts. **a**, The vectors constructed for *in vitro* transcription of the hGR cDNA sequence. The complete α (pGR107) and β (pGR108) coding sequences were placed under the transcriptional control of the SP6 promoter in pGEM1. Vector sequences, noncoding cDNA sequences and coding sequences are indicated by thin lines, thick bars and boxed regions, respectively. The poly(A) tract of ~60 nucleotides is indicated by A_n. Divergent coding sequences are indicated by striped and stippled regions. **b**, Western blot analysis of *in vitro* translation products and cell extracts. Unlabelled translation products synthesized in a rabbit reticulocyte lysate system with no added RNA (lane 1) or with RNA synthesized from pGR108 (β , lane 2) or pGR107 (α , lane 3) were fractionated on a 7.5% SDS-polyacrylamide gel. Additional lanes are: cytoplasmic extracts from IM-9 (lane 4), IM-9 treated with 1 μ M triamcinolone acetonide (lane 5), HeLa (lane 6), ADR6.M1890.AD1 mouse lymphoma (lane 7), S49 mouse lymphoma (lane 8) and EL4 lymphoma (lane 9). Proteins were transferred to nitrocellulose and probed with anti-hGR antibody, followed by ¹²⁵I-labelled *Staphylococcus aureus* protein A as described previously⁴².

Methods. To construct an expression vector containing the entire α coding sequence shown in Fig. 2, the 3' coding sequence of OB7 was fused to OB10 5' coding information. OB7 was partially digested with *Eco*RI, completely digested with *Xba*I, and the 1.2-kbp fragment was gel-purified and ligated with *Eco*RI/*Xba*I-digested OB10 to produce the intermediate pOB107. The entire pOB107 cDNA sequence including the 5' poly(G) tract (11 nucleotides, nt) and 3' poly(A) tract (~60 nt) was excised by partial *Pst*I/complete *Bam*HI digestion. The resultant 3.5-kb fragment was gel-purified and inserted between the *Pst*I and *Bam*HI sites of pGEM1 (Promega Biotec) to yield pGR107. Plasmid GR108 was directly constructed from pOB10 by partial *Pst*I/complete *Bam*HI digestion and insertion of the resulting cDNA insert into the corresponding sites of pGEM1. Capped SP6 transcripts were synthesized from *Pvu*II-linearized pGR107 and pGR108, as described by Krieg and Melton⁶², with simultaneous capping effected by reduction of the GTP concentration from 400 to 100 μ M and addition of m⁷GppG (Pharmacia) to 500 μ M. Transcripts were purified by P60 chromatography and translated with micrococcal nuclease-treated rabbit reticulocyte lysate (Promega Biotec) in conditions suggested by the manufacturer. Preparation of IM-9 cytosol from steroid-treated cells was as described previously⁴². Size markers are phosphorylase B (97K), bovine serum albumin (66K) and ovalbumin (45K).

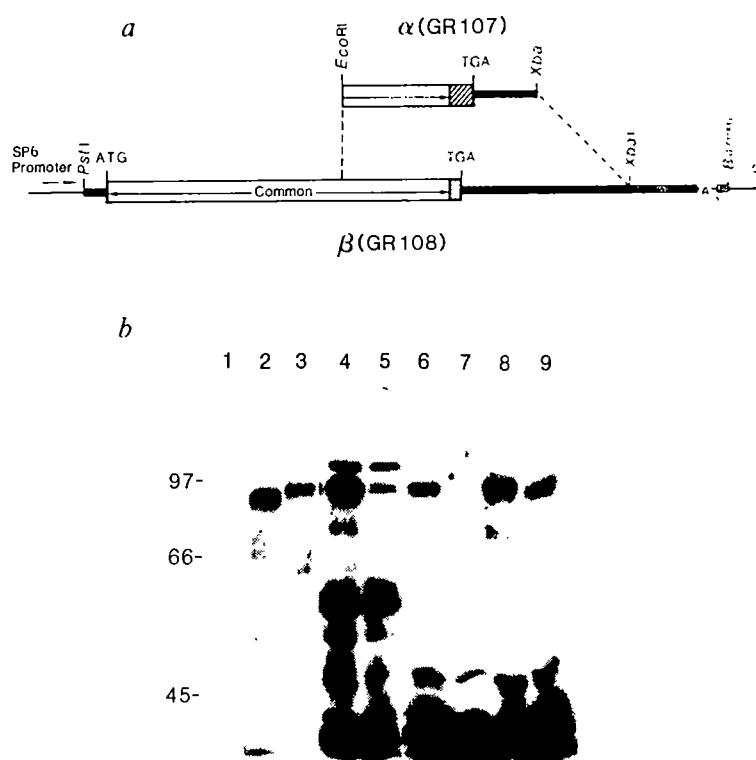
tor cDNA clones and receptor protein by immunoblot analysis, we conclude that the predominant physiological form of the glucocorticoid receptor is the α (94K) species.

Expression of hGR *in vitro*

To provide additional evidence that the cloned receptor is functional, we investigated the possibility that the *in vitro*-translated products might be able to selectively bind corticosteroids. Accordingly, the rabbit reticulocyte lysate was incubated with the radiolabelled synthetic glucocorticoid analogue ³H-triamcinolone acetonide (³H-TA) before or after addition of *in vitro*-synthesized α or β hGR RNA. As shown in Fig. 5, those lysates programmed with α -hGR RNA acquired selective steroid-binding capacity; unexpectedly, the β -receptor synthesized *in vitro* failed to bind competable ³H-TA. The *in vitro*-synthesized α -hGR bound radiolabelled steroid which could be competed with by addition of excess unlabelled cortisol or dexamethasone; however, binding of ³H-TA was not effectively competed with by addition of excess unlabelled oestrogen or testosterone. In contrast, excess progesterone constituted an effective competitor, consistent with the previously reported anti-glucocorticoid activities of progesterone⁴⁷. To confirm these results, the competition experiments were repeated with native glucocorticoid receptor prepared from extracts of human lymphoid cells. Both the *in vitro*-translated receptor and the natural *in vivo* receptor have nearly identical properties with regard to steroid binding and competition with excess unlabelled steroid analogue (Fig. 5).

hGR sequences map to at least two genes

The human glucocorticoid receptor gene has been functionally mapped to chromosome 5. Analysis of somatic cell hybrids constructed by fusing receptor-deficient mouse T cells (EL4)



with human receptor-containing T cells (CEM-C7) indicated that segregants expressing the wild-type CEM-C7 receptor maintained human chromosome 5 while dexamethasone-resistant segregants had lost this chromosome⁴⁸.

To confirm the authenticity of our cDNA clones, we mapped receptor cDNA sequences using Chinese hamster/human somatic cell hybrids containing only human chromosome 5 (HHW454). DNAs extracted from human placenta, HHW454 hybrid cells and Chinese hamster ovary (CHO) cells were digested with *Eco*RI or *Hind*III restriction endonucleases and separated on a 0.8% agarose gel. DNA fragments transferred to nitrocellulose were probed with a portion of the receptor-coding region derived from nucleotides 570-1,640 (hGR1.2A; Fig. 1). In addition to CHO-specific *Eco*RI bands of 6.8 and 17 kbp (Fig. 6a, lanes 2,3), DNA from the hybrid cell line also contains human-specific bands of 3.0 and 5.0 kbp. Unexpectedly, a DNA fragment of 9.5 kbp is found in total human DNA but not in the hybrid line (Fig. 6a, lane 1). Similarly, *Hind*III digestion revealed a 7.5-kbp band that is not present in the chromosome 5 hybrid cell DNA (Fig. 6a, lane 4). These results indicate that the receptor cDNA maps to human chromosome 5, but that there are additional receptor-related sequences elsewhere in the genome. To map these sequences, we used a dual-laser fluorescence-activated cell sorter (FACS) to sort mitotic chromosome suspensions stained with DAPI/chromomycin in conjunction with Hoechst 33258 chromomycin; this technique allows separation of the 24 human chromosome types into 22 fractions⁴⁹. After the chromosomes were sorted directly onto nitrocellulose, the chromosomal DNA was denatured and hybridized to the hGR cDNA probe. In addition to confirming the chromosome 5 localization, additional sequences were found on chromosome 16 (Fig. 6b). To confirm this localization, DNAs from mouse erythroleukaemia cells and a mouse erythroleukaemia cell line containing human chromosome 16 (ref. 50) were digested with

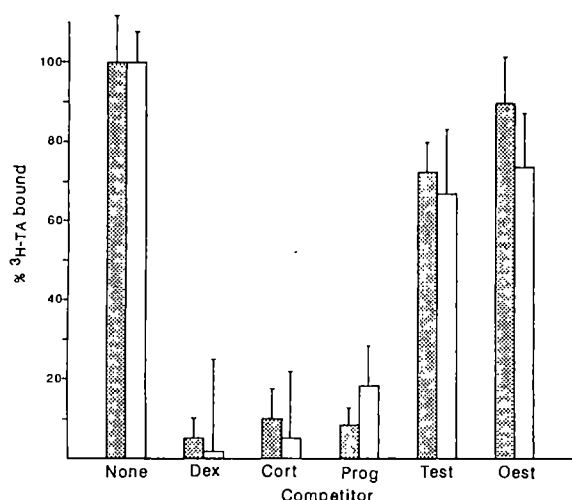


Fig. 5 Steroid-binding of α -hGR (GR107) translated *in vitro*. Binding to IM-9 cytosol extract (stippled bars) and to reticulocyte lysate containing SP6-generated α -hGR RNA (GR107; open bars) are shown. Bars represent bound ^3H -triamcinolone acetonide (TA) determined with a 100-fold excess of various steroid competitors; 100% competition was determined using unlabelled TA as competitor. The values represent the mean of triplicate determinations, with error bars showing $P < 0.05$. Steroid competitors are dexamethasone (Dex), cortisol (Cort), progesterone (Prog), testosterone (Test), and oestradiol (Oest).

Methods. Binding assays were performed in 100 μl containing 10 mM Tris-HCl pH 7.4, 100 mM NaCl, 1 mM EDTA, 10 mM sodium molybdate, 10 dithiothreitol, 150 mM ^3H -TA (20 Ci mmol $^{-1}$; Amersham) and 10 μl translation mixture or 100 μg fresh IM-9 cytosol. Unlabelled steroid competitor (15 μM) was added as indicated. After 2 h at 0 $^{\circ}\text{C}$, samples were extracted twice for 5 min each with 5 μl of 50% dextran-coated charcoal to remove unbound steroid, and counted. Uncompeted and fully competed values for the α glucocorticoid receptor (GR107) were 490 and 290 c.p.m., respectively. Reticulocyte lysate translation mixtures without added transcript or programmed with β -receptor SP6 RNA (GR108) contained no competeable ^3H -TA binding.

*Hind*III and probed with hGR cDNA (Fig. 6c); as predicted, the only DNA fragment found in the hybrid and not in the control was the 7.5-kbp DNA fragment, thus establishing the chromosome 16 assignment (Fig. 6c, lanes 1, 2).

Additional Southern blot analyses using the *Eco*RI-*Xba*I fragments from OB7 and OB10 3'-untranslated regions revealed hybridization only to chromosome 5 (data not shown). We conclude that both the α - and β -receptor cDNAs are probably encoded by a single gene on chromosome 5 and suggest that the two cDNA forms are generated by alternative splicing. In addition, we conclude that another gene residing on human chromosome 16 contains homology to the glucocorticoid receptor gene, at least between nucleotides 570 and 1,640. It is not clear whether these sequences on chromosome 16 represent a related steroid receptor gene, a processed gene or pseudogene, or a gene that shares a common domain with the gene for the glucocorticoid receptor. Genomic cloning and DNA sequencing may provide the answer.

To determine the size of the mRNA encoding the glucocorticoid receptor, Northern blot hybridization⁵¹ experiments were performed using cytoplasmic mRNA isolated from a human fibroblast cell line, HT1080. Using the hGR1.2 coding sequence as probe, multiple mRNAs of 5.6, 6.1 and 7.1 kb were detected. Treatment of these cells with glucocorticoids for 24 h leads to a 2-3-fold reduction in receptor mRNAs, suggesting a potential negative feedback regulation.

Discussion

Structural analysis of the glucocorticoid receptor is a prerequisite for gaining insight into the mechanisms by which this regulatory molecule exerts its effects on gene transcription. Here, we have presented the primary sequence of the human glucocorticoid receptor deduced from nucleotide sequence analysis of cDNA clones.

Isolation of hGR cDNAs has revealed the existence of multiple mRNAs encoding at least two forms of the polypeptide. The predicted proteins differ at their carboxy termini by the substitution of 50 amino acids in the case of α -hGR and 15 amino acids in the case of β -hGR. The α glucocorticoid receptor is the major

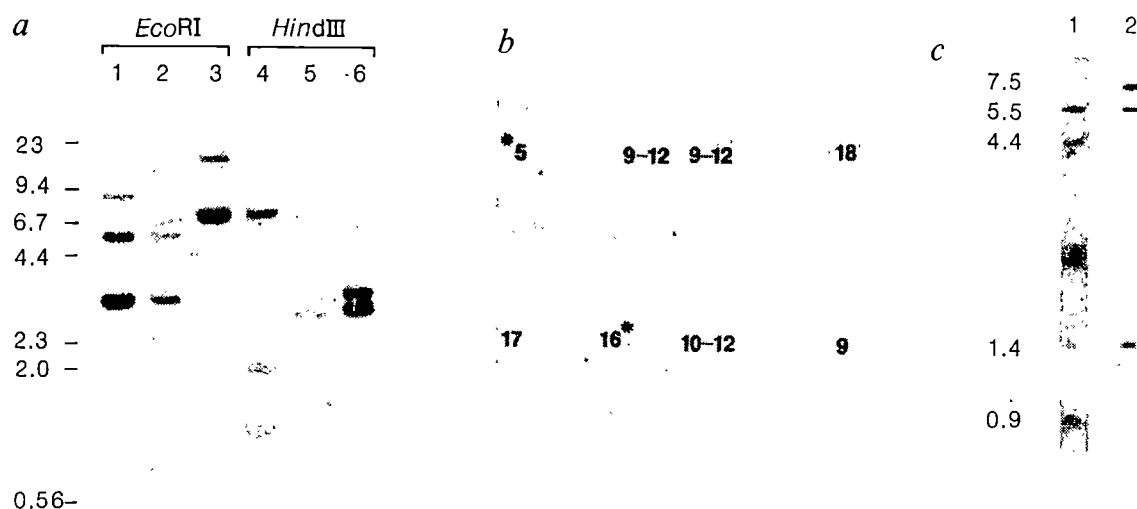
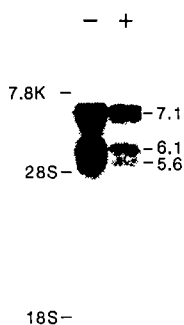


Fig. 6 Chromosome mapping analysis of hGR cDNA. **A**, 10 μg of DNA from human placenta (lanes 1, 4), CHO/human somatic cell hybrid (HHW454, lanes 2, 5) containing chromosome 5 as its only human complement, or CHO (lanes 3, 6), was digested with *Eco*RI (lanes 1-3) or *Hind*III (lanes 4-6) to completion, fractionated on a 0.8% agarose gel and transferred to nitrocellulose paper. **B**, Chromosomes (3×10^4) prepared from a human lymphocyte cell line, stained with 4,6-bis(2"-imidazolyl)-4H,5H)-2-phenylindole (DIPI)/chromomycin A3 and sorted using a dual-laser custom FACS IV chromosome sorter⁶³, were denatured and neutralized on nitrocellulose paper. Note that Hoechst/chromomycin-stained chromosome 9 was sorted with chromosomes 10-12. **C**, 10 μg of DNA from the parental mouse cell line MEL (lane 1) or the parentally derived somatic cell hybrid carrying human chromosome 16 (ref. 50; lane 2) was digested with *Hind*III and separated on a 0.8% agarose gel, then transferred to nitrocellulose paper. All filters were probed with the 1,100-bp insert from hGR1.2, nick-translated to a specific activity of 3×10^8 c.p.m. μg^{-1} and hybridized in $5 \times \text{SSPE}$, $1 \times \text{Denhardt's}$, 0.1% SDS, 50% formamide, 100 $\mu\text{g ml}^{-1}$ denatured salmon sperm DNA, 50% dextran sulphate at 42 $^{\circ}\text{C}$ for 18 h. Filters were washed twice (for 30 min each) in $2 \times \text{SSC}$ at 68 $^{\circ}\text{C}$ and exposed to X-ray film at -70 $^{\circ}\text{C}$ with an intensifying screen.

Fig. 7 Northern blot analysis of hGR mRNA. 10 μ g of poly(A)⁺ mRNA from human HT1080 fibroblast cells, collected after 24 h without (–) or with (+) treatment with 10 μ M dexamethasone, was electrophoresed through a 0.8% agarose/1% formaldehyde gel, stained with acridine orange (120 μ g ml^{–1}) and transferred to nitrocellulose. The filter was hybridized overnight with nick-translated hGR1.2 (10⁶ c.p.m. ml^{–1}, specific activity 10⁸ c.p.m. μ g^{–1}) and washed with 2 \times SSC at 68 °C. Sizes were estimated from human fibronectin mRNA (7.8 kb), and 28 S (5.0 kb) and 18 S (2.1 kb) ribosomal RNAs.



form identified in several human cell lines and cDNA libraries. However, a recent report by Northrop *et al.* characterizes two forms of the receptor in mouse lymphoid cells⁵². The relationship of α - and β -hGR to the mouse doublet species remains to be established. Also, the cellular distribution and potential function of β -hGR are unclear, although it is possible that variant receptors are used for tissue-specific functions. We are now generating antisera to synthetic peptides specific for each human receptor form to elucidate their tissue-specific expression.

Among the cDNAs selected using the immunopositive phage DNA insert hGR1.2A as probe were those containing 3' ends similar to OB7, except that polyadenylation was signalled earlier by the use of an AATAAA at nucleotide 3,101. These clones have been isolated from both human fibroblast and placental libraries (data not shown). Alternative poly(A) site selection is a feature of many eukaryotic transcription units⁵³. In some instances, selection of poly(A) sites specifies particular polypeptide products^{54–57} while in other cases, alternative poly(A) site selection produces no change in the primary structure of the polypeptide⁵⁸. The selection of poly(A) sites during receptor transcription may (1) alter the stability of the mRNA in a particular tissue, (2) lead to splicing changes, or (3) be random, with no physiological consequence.

The *in vitro* translation studies described here provide direct evidence that the cloned molecule encodes the complete glucocorticoid receptor. First, the *in vitro*-translated product is identical in size to the native glucocorticoid receptor and is immunologically reactive with receptor-specific antiserum.

Second, the *in vitro*-translated protein acts functionally as a glucocorticoid receptor in that it is capable of selectively binding the synthetic glucocorticoid triamcinolone acetonide. This binding is specifically competed with by glucocorticoids, glucocorticoid analogues and progesterone but is not competed with by the sex steroids testosterone and oestrogen. In this respect, the *in vitro*-translated receptor behaves identically to the *in vivo* receptor from human lymphoid cells, providing the first evidence for a function of the cloned molecule. The acquisition of steroid-binding properties does not appear to require any specific modifications or, if it does, these modifications can occur in the *in vitro* translation mix.

The results presented here provide the information necessary for studying the molecular interactions of a eukaryotic transcriptional regulatory protein with its target genes. These structural studies provide a basis from which the glucocorticoid receptor, its gene, and its RNA products can be analysed. Furthermore, the ability to express receptor *in vitro* provides a novel means by which the consequence of specific *in vitro* mutagenesis can be rapidly tested.

In addition to the *in vitro* studies, the analysis of several existing rodent cell lines^{49,59–61} with well-characterized receptor defects in both the DNA- and steroid-binding domains should facilitate future analysis. Furthermore, the isolation of genes responsive to glucocorticoids and specific regulatory elements by both mutagenic and protein-binding studies suggests that this protein will serve as a very useful model for analysis of inducible eukaryotic gene regulation.

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A 12.6-ms pulsar in Cygnus X-3

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The X-ray binary Cygnus X-3 has long been suspected of containing a fast, young pulsar, but previous observations at various wavelengths have failed to provide any evidence of a signal with a periodicity of less than 4.8 h (the well-known periodicity in the X-ray and infrared wavebands which is ascribed to orbital motion of a binary system). Here we report measurements of 1,000-GeV γ -rays which suggest a pulsar period of 12.5908 ± 0.0003 ms, for data recorded in 1983 at the times of X-ray maximum in the 4.8-h cycle. The conservative probability of such a periodicity occurring by chance is 4×10^{-7} and, moreover, supportive evidence is provided by some earlier data.

Cyg X-3 has been observed many times by γ -ray telescopes at TeV energies¹⁻⁵ and more recently by cosmic-ray detectors at PeV energies⁶⁻⁷. There is a consensus that the TeV emission is confined to two short periods in the 4.8-h X-ray modulation cycle⁸, with the stronger period occurring about X-ray phase 0.625. Previous measurements using the Dugway array of four telescopes¹ have indicated that this emission may last for only ~ 10 min. Until 1983, the gross counting rate of the telescope array was insufficient to allow a search for a possible periodicity.

Observations using improved detectors were made in August–October 1983, with Cyg X-3 being continuously tracked for long intervals under a wide range of weather conditions. The best atmospheric conditions were encountered during a 6-h observation made on 1983 September 12, with ideal weather. The time of arrival of each event was recorded with a resolution of 1 μ s and an absolute uncertainty of ~ 0.5 ms. Each event time was adjusted to Ephemeris time at the solar system barycentre using the MIT ephemeris⁹. At 0519 UT the 4.8-h X-ray phase was 0.625. At this phase a count rate excess was noted which lasted for ~ 7 min. The most important feature of this 7-min interval is that it offers, for the first time, a sample of sufficient size and signal strength to make a pulsar period search worthwhile.

We have tested the 450 events contained in the 7-min interval for periodicity over the range 10 ms to 50 s, using the most appropriate test for the power in the fundamental—the Rayleigh test¹⁰. The period with by far the smallest probability of a chance origin was 12.5908 ms, the probability being 4.8×10^{-8} (see Fig. 1a). Allowing for the number of periods tested, the true significance of this periodicity is 3.3×10^{-3} or 3σ . Although in itself not particularly significant, this possible periodicity has the support of further independent evidence.

First, we have considered the possible correlation between counting rate and periodicity within the 7-min interval. If the periodicity is genuine, the onset and decay of the count rate excess should, on average, be mapped by the variation in the fraction of the counts which are periodic. In the null case (that the apparent periodicity is just a random fluctuation) the fraction of counts which are apparently periodic should be independent of the actual number of counts, hence there should be no correlation between these values. As the joint probability of a segment of the 7-min interval having a particular fractional periodicity and also having a particular gross number of counts (given the null hypothesis of no real periodicity) can be factorized, a correlation would provide independent evidence of periodicity¹¹. Although, formally, product moment correlation requires normally distributed samples, in practice a wide variation of sampling distributions is found to be permitted^{12,13}. We find that the strength of the 12.5908-ms periodic component

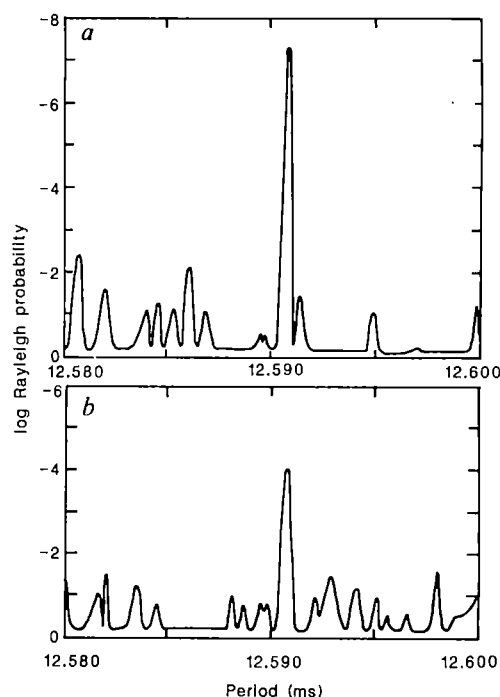


Fig. 1 *a*, The chance probability for periodicity in the 450 events containing the count rate excess on 1983 September 12, as a function of trial period. *b*, The chance probability of periodicity for the events on 1983 October 2 at precisely the same phase in the 4.8-h cycle as was investigated on 1983 September 12, as a function of trial period.

correlates (at a significance level of 9.5×10^{-4} or 3.1σ) with the count rate in independent 1-min data segments within the 7-min interval. The correlation arises mainly from two of the minutes (which are 3 min apart and are roughly centred in the 7-min interval) during which both the counting rate and the periodic signal are both particularly strong.

Second, we examined other nearby data sets containing precisely the same phase in the 4.8-h period. During August–October 1983, seven other such observations of Cyg X-3 were made. In no case were the climatic conditions stable enough to allow reliable use of the minute-by-minute count rates to unambiguously identify a count rate excess. However, tests for millisecond periodicity at a known period are possible in such data. For one of the seven observations (1983 October 2) exactly 100 4.8-h cycles after the detection on September 12, a periodicity was observed in the 7-min interval at 12.5908 ms, with a chance probability of 4×10^{-4} (see Fig. 1b) (the larger chance probability reflects the lower counting rate due to non-ideal climatic conditions during the October measurements).

The interpretation of this second occurrence of periodicity is complicated by the possible presence of two 19-day periodic effects: a 19.3-day amplitude modulation⁵ of the TeV emission at X-ray phase 0.625 based on our 1981/82 data, and the 18.7 day phase modulation of amplitude ± 3 min in the time of the X-ray minimum found by the COS-B X-ray detector¹⁴ and attributed to apsidal motion. If the 19.3-day amplitude modulation is genuine, and since the excess on September 12 was near a 19.3-day peak, the October 2 observation would be the one expected to show the most significant periodic excess. Because of the 18.7-day phase modulation, the 20-day separation of our two observations may produce up to a minute in phase difference. We note that data in a 7-min interval centred 50 s later than the precise time predicted from the X-ray ephemeris and our previous measurement, show periodicity at 12.5908 ms with a smaller chance probability, that is, 4×10^{-5} .

To be conservative, we have (1) allowed degrees of freedom for all seven observations, ignoring any possible 19.3-day amplitude modulation (although this is noted for future confirmatory observations) and (2) ignored the possible improvement in

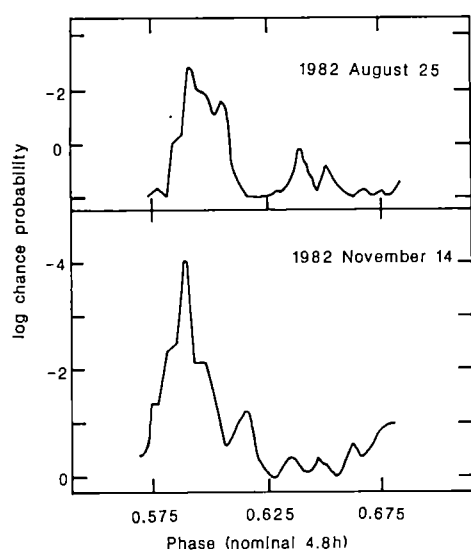


Fig. 2 The chance probability of periodicity (12.5908 ms) as a function of phase in the nominal 4.8-h period, for observations on 1982 August 25 and November 14.

chance probability by shifting the time of the 7-min interval to allow for possible apsidal motion. This leads to a chance probability of 2.8×10^{-3} for detecting a 12.5908-ms periodicity on any one of the seven occasions.

Finally, we have demonstrated previously that those events which trigger two separate telescopes spaced 60 m apart (twofold events) have a narrower acceptance angle distribution than is the case for events registered in a single telescope¹⁵. A point source of γ -rays centred in the field of view will consequently have a proportionally greater effect on the rate of cosmic-ray background detected as twofold responses than is the case for single-telescope responses. In the present observation, the fraction of the twofold events showing periodicity was 1.5 times greater than that of the single-telescope events, in accord with our previous experience. However, the restricted sample size gives an uncertainty of ± 0.5 in this value so that, in this case, formal use cannot be made of the increased sensitivity of the two-fold responses.

Although the general periodicity search was terminated at 10 ms because of the possible loss of coherence for shorter periods, periods near integral factors of 12.5908 ms have been searched for in shorter segments of the 7-min interval. No evidence was found to indicate that 12.5908 ms is a multiple of a shorter periodicity.

Our data for 1983 thus provide chance probabilities of: (1) 3.3×10^{-3} (September 12 periodicity allowing for all periods tested); (2) 9.5×10^{-4} (correlated periodicity and count rate on September 12); and (3) 2.8×10^{-3} (periodicity on October 2 at precisely the predicted phase in the 4.8-hr cycle, allowing for non-detection of signals from six other data sets). These three probabilities are derived from independent data or independent tests on the same data and may therefore be combined. The combined probability of 4×10^{-7} is conservative.

To check for possible instrumental effects, off-source data from the same and adjacent nights were analysed similarly and the Rayleigh test probability distributions are found to be those expected by chance, as is the distribution during the 7-min excess, discounting the 12.5908-ms peak. The 12.5908-ms periodicity was detected in each of the four well-separated (100 m) telescopes which were operated and triggered independently, largely excluding the possibility that local pick-up caused the increase in counting rate. Also, more data were available on each event other than the time of arrival (UT). The relative times of triggering of each detector were recorded with 150-ps resolution. For those events which triggered two or more detectors, the correct time separation was observed for Cerenkov light flashes in the field of view. The pulse height in all three of the photomultipliers on each detector was also recorded for each

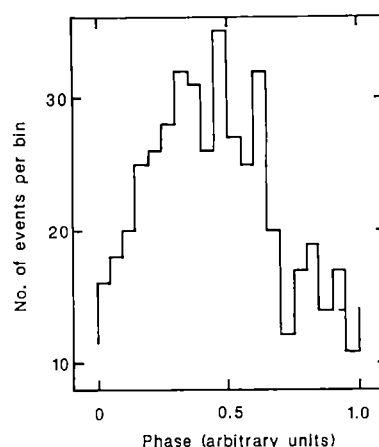


Fig. 3 Light curve for the TeV γ -ray emission during the 7-min data set at X-ray maximum on 1983 September 12.

event, and the spectrum during the 7-min interval was that expected for Cerenkov flashes. All the anode currents and photoelectron noise trigger rates of the individual photomultipliers were also recorded and were normal during the 7-min interval. Therefore, we believe that the events giving rise to the periodic excess were genuine Cerenkov light flashes in the field of view.

We have examined our 1982 data for instances of similar periodicity. In 1982 the counting rates were considerably lower than in 1983, and we would not expect any significant contribution to the overall chance probability of periodicity from an interpretation of these data. Furthermore, the absolute phase in the 4.8-h orbit of the data to be tested will have uncertainty due to variations arising from possible apsidal motion and from errors in the ephemeris for the 4.8-h period. The uncertainties in the 4.8-h period suggest variations of ~ 5 –10 min in the phase in 1982 relative to that in 1983. We have therefore passed a window of 7 min duration through the data, incrementing the start time by 1-min steps spanning ± 15 min from the nominal phase 0.625. The nominal 4.8-h orbit uses the van der Klis and Bonnet-Bidaud⁸ ephemeris incorporating a period derivative. Each data set was tested for periodicity at 12.5908 ms. There is evidence for periodicity during 2 of the 14 observations (compared with 2 out of 8 in 1983). The strength of the periodicity as a function of the nominal 4.8-h phase on these two occasions is shown in Fig. 2. These two detections concur in the emission being earlier than the nominal phase. There is thus support for 12-ms periodicity from the 1982 data. Our 1981 data were taken in drift-scan mode with detectors of lower counting rate and less precise millisecond timekeeping and thus are less suitable for this analysis.

If the 4.8-h X-ray modulation is due to an orbit, as is generally concluded, the sections of data suggesting periodicity and lasting for 7 min should show some evidence of an orbitally derived period derivative. The periodicity test has been relaxed for a range of values of period derivative between -10^{-8} and $+10^{-8} \text{ s s}^{-1}$. The combined probability for the two positive data sets in 1983 was found to minimize for a small negative derivative: $-1.2 \pm 0.7 \times 10^{-9} \text{ s s}^{-1}$. A similar treatment of the two data sets in 1982 gave a derivative of $-0.6 \pm 0.4 \times 10^{-9} \text{ s s}^{-1}$. These estimates combine to give $-7.5 \pm 3.5 \times 10^{-10} \text{ s s}^{-1}$. This negative value of derivative places the source of the TeV emission, which coincides with the X-ray maximum, in the rear half of the orbit. Because the TeV emission is at X-ray maximum, this result (which is significant only at the 2σ level) conflicts with the two main models for the X-ray modulation—the cocoon model and the stellar wind model. In these models the X-ray emitter is in front of the companion at X-ray maximum. Both models assume the X-ray source is an accreting neutron star or a pulsar and the X-ray modulation is due to variations in the absorption or scattering, and both models require a significant orbital eccentricity. However, the sign of the derivative is consistent with a TeV-emission model¹⁶ and places the source of the very-high

energy ions behind the companion's atmosphere at the time of TeV emission. With the assumptions that TeV γ -ray emission occurs when the pulsar is almost directly behind the companion, and that the orbit is nearly circular (as inferred from the COS-B observations of the 18.7-day X-ray phase modulation), the observed period derivative of $-10^{-9} \text{ s s}^{-1}$ would correspond to an orbital velocity of $\sim 80 \text{ km s}^{-1}$. In this case the Keplerian mass function would be $\sim 0.01 M_{\odot}$.

Recently, two models have been proposed to explain the production of TeV and PeV γ -rays from Cyg X-3: (1) the luminosity is derived from the rotational energy of a rapidly spinning ($\sim 10 \text{ ms}$) neutron star of high magnetic field¹⁶, and (2) acceleration of ions occurs in an accretion disk surrounding a neutron star by the unipolar inductor mechanism¹⁷. Both models require the high-energy ions to interact with the atmosphere of the companion to produce the γ -rays. The light curve for the TeV emission during the 7-min interval on 1983 September 12 (for a period of 12.5908 ms and including the period derivative) is broad (see Fig. 3). The curve for the emission on October 2 is similar, and both curves have the same form as the TeV light curves of other X-ray binaries (Hercules X-1¹⁸; 4U0115¹⁹) and the 6-ms pulsar PSR1953+29²⁰. This is in contrast to the very sharp light curve in the case of an isolated pulsar—the Crab²¹. This suggests that accretion from a pulsar's binary companion plays an important part in the TeV emission.

The TeV γ -ray flux appropriate to this observation is consistent with our previous measurement¹ of $10^{-39} \text{ W m}^{-2} \text{ Hz}^{-1}$. The differential power-law spectral index of ~ 1 implicit in a comparison of this flux with the X-ray flux suggests that TeV γ -rays constitute the majority of the electromagnetic emission from Cyg X-3. The total energy flux, over all wavelengths, has been estimated at $\sim 10^{38} \text{ erg s}^{-1}$; this, together with a pulsar period of 12.5908 ms, would require a spin-down period derivative, given a magnetic field similar to that of the Crab pulsar, of about $10^{-14} \text{ s s}^{-1}$. On the other hand, an accretion-driven low-magnetic-field system may be in equilibrium or may be spinning up. In either case, the change in period expected between the observations in 1983 and those in 1982 would be $< 0.3 \mu\text{s}$. Our sampling uncertainty in period is about the same, precluding any measurement of a secular period change until a precise orbit has been determined.

Thus, we have prima facie evidence from our 1983 data, at a chance probability of 4×10^{-7} , that the excess counts of 1,000-GeV γ -rays noted from Cyg X-3 at orbital phase 0.625 in the 4.8-h X-ray cycle are all periodic, with a period in the orbit in 1983 September/October of $12.5908 \pm 0.0003 \text{ ms}$. Other qualitative evidence from observations in 1983 and 1982 supports this suggestion. However, further observations of Cyg X-3 are required to confirm the existence of this periodicity. Note that for all other wavelength bands where Cyg X-3 has been detected, current models would predict that such a millisecond pulsar periodicity would be greatly attenuated due to dispersion or scattering in the cocoon or dense stellar wind surrounding the companion. However, rapid quasi-periodic oscillations reported recently²² from GX5-1 have been interpreted^{22,23} as beating between the period of a fast pulsar and the orbital period of the inner edge of the accretion disk. Such indirect effects of a 12.59-ms pulsar may be detectable, or may be involved in the longer-period quasi-periodic oscillations already detected from Cyg X-3 (ref. 24).

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Optical polarization observations of circumsolar dust during the 1983 solar eclipse

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It is very difficult to make optical observations from the Earth where the angular distance from the Sun is greater than $3 R_{\odot}$ (where R_{\odot} is the angular radius of the Sun) because of the large and uncertain correction for sky radiation. To overcome these difficulties, we have now made optical polarization observations at four wavelengths (5,330 Å, 5,970 Å, 7,200 Å, 8,015 Å) for the outer solar corona at an altitude of 30 km, using a balloon-borne silicon-intensified (SIT) TV camera during the total solar eclipse on 11 June 1983 in Indonesia. As reported here, a polarization excess at an elongation between 4 and $5 R_{\odot}$ (1° and 1.25°) from the Sun in the ecliptic plane was observed at a wavelength of 8,015 Å on the two-dimensional frame. This is interpreted as an enhancement of dust grains distributed in a ring around the Sun approximately in the ecliptic plane.

Infrared excesses have been observed¹⁻⁵ at the elongation around 4 and $10 R_{\odot}$ which have confirmed the existence of a

Table 1 Polarization distribution at the region of enhancement

θ/R	3.6	3.8	4.0	4.2	4.4	4.6	4.8	5.0	5.2	5.4
South										
16	15	14	14	13	11	10	8	9	8	8
14	14	15	14	14	12	11	9	8	6	6
12	14	16	15	14	12	11	10	8	7	6
10	14	17	16	14	13	11	11	9	6	6
8	14	17	17	15	14	11	10	9	6	6
6	14	17	17	16	15	11	9	9	7	6
4	12	17	18	17	14	13	10	9	8	7
2	12	18	19	17	15	13	13	9	8	7
North										
0	12	18	19	16	14	14	12	9	8	8
2	12	17	19	17	15	14	11	10	8	7
4	0	17	18	18	16	14	12	9	8	8
6	0	17	19	17	15	14	13	10	9	8
8	0	17	20	18	16	14	12	10	9	7
10	0	16	20	17	16	14	12	10	8	8
12	0	15	19	16	15	11	11	11	8	7
14	0	14	19	17	16	15	11	10	8	7
16	0	13	19	17	15	14	10	10	7	6

R is the angular distance from the Sun in units of R_{\odot} . θ is the angle of radial line from the Sun to the ecliptic. $\theta = 0^{\circ}$ is on the ecliptic and $\theta = 10^{\circ}$ in the north is on the solar equator. At $R = 3.6 R_{\odot}$, data are probably affected by the occulting disk.

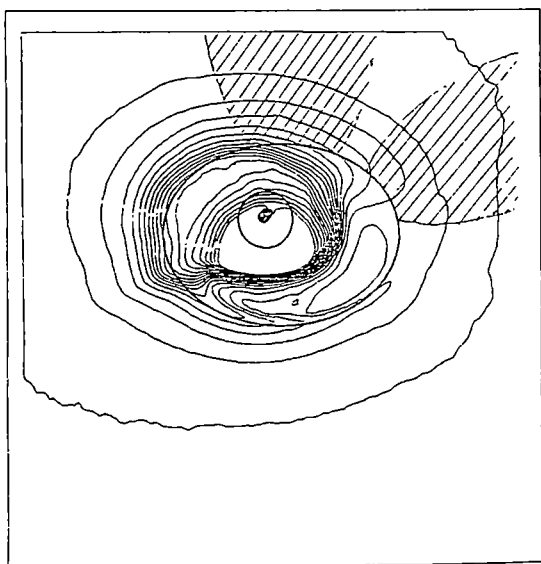


Fig. 1 An intensity contour map at a wavelength of 8,015 Å. The small circle and the large ellipse are the positions of the Sun and the occulting disk, respectively. The two areas suffering ghost images are hatched. The contour unit is arbitrary. The field-of-view is $5^\circ \times 5^\circ$ corresponding to $20 \times 20 R_\odot$. North is down and west is left.

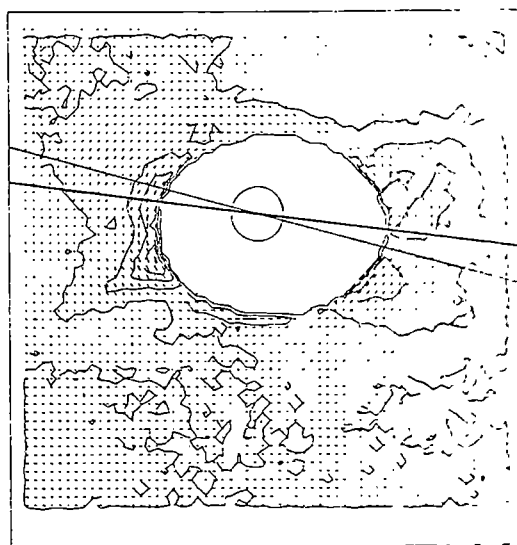


Fig. 2 A contour map of polarization degree in a field of $5^\circ \times 5^\circ$. Contour unit is 5%. The ecliptic plane and solar equator are shown by thick and thin lines, respectively. North is down and west is left. The length and direction of each short line denote degree of polarization and direction of electrical vector, respectively. Note that there is some effect of the ghost images in the areas shown in Fig. 1.

dust-free zone inside the solar distance (radial distance from the Sun) at about $4 r_\odot$ (r_\odot is the linear radius of the Sun), and indicates that there is a ring-shaped enhancement in the density of dust grains along the outer edge of this zone. To observe these areas, we used an optical telescope with a field of $5^\circ \times 5^\circ$ ($20 \times 20 R_\odot$) centred almost on the Sun. Because our SIT TV camera has 256×256 pixels, the observed resolution is $0.08 R_\odot$. Apart from the introduction of a small shift of the Sun (10 arc min to the south-west; upper left in Fig. 1) from the centre of the occulting disk to eliminate bright light from the inner corona, the observations were successful. Unfortunately, the shift had severe saturation effects on the observations at 5,330 Å, 5,970 Å and 7,200 Å, and therefore only the observational data at 8,015 Å are presented here. All the data, including those at the other wavelengths, will be published elsewhere^{6,7}.

During the 140 s of the observation, we rotated two wheels: the filter wheel was kept fixed for 15 s (in each filter) while the polarizer wheel was rotated to each of the eight positions, remaining in each position for 1.8 s. We thus have two complete sets of polarization observations at the four wavelengths. All observational data were recorded by a video recorder on-board the balloon. After recovery of the tape, all video data were transferred to digital data using a computer with a 16-bit double-frame memory. To control for the non-uniformity of the detector sensitivity of the SIT TV cameras and the instrumental polarization created by our telescope system, artificial uniform unpolarized light was measured. After correction, the error of polarization at each point of the detector was estimated to be $\pm 1\%$. Although the asymmetrical position of the Sun in the TV frame made two ghost images (see Fig. 1), there was no effect on the other areas in the field.

Figure 2 shows a contour map of the degree of polarization, where the length and direction of each line denote the degree of polarization and the direction of the electrical vector, respectively. The polarization vector should be perpendicular to the radial direction from the Sun, because coronal light is scattered by coronal electrons (K corona) and interplanetary dust (F corona). Most vectors shown in Fig. 2 satisfied this condition. The numerical values of the degrees of polarization in the areas of interest are shown in Table 1 and indicate a smooth variation of polarization between successive points. It has been difficult to separate the two components of the F and K corona in the observed intensity, for the only way to separate them is to

observe the depth of Fraunhofer lines in the spectrum of the corona and to compare them with the depth in the solar spectrum⁸. We have observed the total intensities of the combined F and K coronal components and so could obtain no information on their relative proportions. Van de Hulst⁹ collected data on the F and K coronal components between 1 and $10 R_\odot$ and showed that the K component is brighter than the F component at an elongation of $< 2 R_\odot$, but decreases to $< 20\%$ of the F component beyond an elongation of $3.5 R_\odot$ except for the area of the coronal stream. Figure 2 shows clearly an area with a high degree of polarization which corresponds to a coronal stream.

We have compared our polarization distribution in the ecliptic plane with other results. Blackwell and Petford⁸ observed the outer corona and obtained the intensity and polarization of the F and K components at an elongation greater than $5 R_\odot$. Following their results, the degree of polarization of total light for the F and K components is $< 8\%$. During the solar eclipse on 5 February 1962, Saito and Hata¹⁰ made an observation of the polarization at 6,200 Å and showed that the degree of polarization decreases between 1.2 and $3.0 R_\odot$ in elongation and that the extrapolated value at an elongation of $5 R_\odot$ is $< 10\%$. Our results show $\sim 17\%$ polarization at an elongation of between 4 and $5 R_\odot$ (see Fig. 3). It is not clear why the previous results did not show high polarization at elongations between 4 and $5 R_\odot$, but we think that photographic observations of polarization such as those of Saito and Hata have a low signal-to-noise ratio at the elongation considered and that photoelectric observations of polarization cover only a small coronal area in each limited observational period during the solar eclipse. Unfortunately, Blackwell and Petford's extensive observations were carried out at large elongations ($5\text{--}16 R_\odot$). Our observations were carried out using a SIT TV camera with as high an accuracy as is obtained with photoelectric observations and, using a two-dimensional frame for the photographic observations, show an area of high polarization (see Figs 2, 3).

If there is an enhancement in the density of dust grains around $4 r_\odot$ as suggested by the infrared observation, the enhanced grains located between 4 and $5 r_\odot$ on the line-of-sight, at an elongation between 4 and $5 R_\odot$, scatter the solar light with a scattering angle of around 90° . The scattered light is polarized by 0–60% depending on the refractive index and radius of dust grains and on the wavelength of the light^{11,12}. Therefore, one

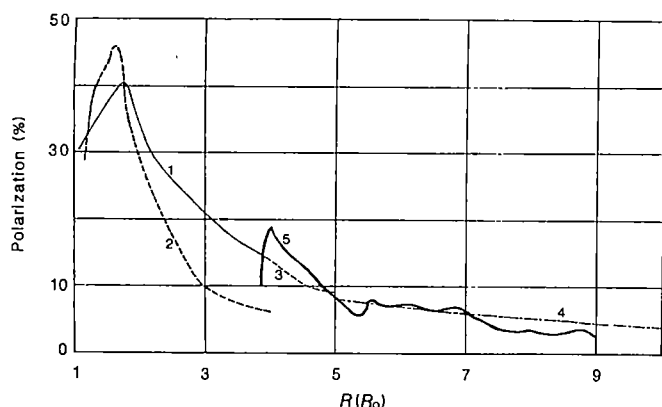


Fig. 3 Degree of polarization depending on the elongation in the ecliptic plane. Thin solid (1) and thick dashed (2) lines are those observed by Saito and Hata¹⁰ in the east and west directions respectively. Thin dashed line (3) is an extrapolation of thin solid line. Dashed-dotted line (4) shows the results of Blackwell and Petford⁸. Thick solid line (5) is our result in the west direction which shows a high degree of polarization at the elongation between 4 and 5 R_{\odot} . There is a jump of polarization from 0 to 15% at the elongation of 3.8 R_{\odot} corresponding to the cutoff point of the occulting disk, but the peak of polarization at 4.0 R_{\odot} is real. The observational error is 1% at the inner region.

can expect a high value for polarization at an elongation between 4 and 5 R_{\odot} in the ecliptic plane as a result of dust grains that have a scattering angle of $\sim 90^{\circ}$. Because there is no enhancement of polarization in the direction perpendicular to the ecliptic plane in Fig. 2, the region with the highest number density of dust grains does not extend far from the ecliptic plane.

We conclude that there is an enhancement of the degree of optical polarization at an elongation between 4 and 5 R_{\odot} which suggests that the number of dust grains distributed in a ring around the Sun increases approximately at the ecliptic plane.

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Decay of the cometary bow shock

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Cooling processes will weaken the bow shock that is expected to form when the solar wind plasma encounters a gassy comet. As the supersonic wind penetrates the comet's outer coma, accreting freshly ionized cometary atoms and molecules, it needs a shock to adjust to the inner subsonic conditions. The cometary ions, implanted in the plasma stream, are accelerated by the associated fields and take up much of the decrease in streaming energy. The

subsonic flow in comets is distinguished by strong cooling, effected primarily through ion-molecule reactions between the energetic implanted ions and the neutral gas coma. We argue here that such cooling can cause complete decay of the shock's flanks, as probed by the ICE (International Cometary Explorer) spacecraft at comet Giacobini-Zinner.

To the on-rushing solar wind, the extensive and rarefied cometary coma presents a very different obstacle from a planetary magnetosphere, or even a planetary exosphere such as Venus. The solar wind plasma penetrates freely through the outer coma, except that associated electric and magnetic fields couple it to occasional freshly ionized cometary atoms and molecules. These implanted cometary ions have commonly been viewed as 'picked up' in the plasma flow, their bulk effect being modelled as sources of mass m , momentum S and energy E added to the gas-dynamic equations for the flow¹. The sources combine to constitute the generalized drag^{2,3}

$$D = \frac{1}{2}(\gamma + 1)m - \gamma S/u + \frac{1}{2}(\gamma - 1)E/u^2 \quad (1)$$

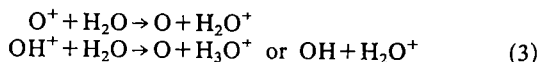
in a flow at speed u , specific heat ratio γ . Evidently, mass-accreting ($m > 0$) and frictional ($S < 0$) contributions to D are offset by cooling ($E < 0$). This drag coefficient D enters the 'throat' equation, crucial for investigating transonic gas-dynamic flows⁴: for a streamtube of cross-sectional area A and length z ,

$$(1 - M^2)du/u = M^2 D dz/\rho u - dA/A \quad (2)$$

where $M = \sqrt{\rho u^2/\gamma p}$ is the Mach number, ρ the density and p the pressure. While flows with 'mass-loading' alone with D everywhere positive have attracted much attention, the presence of an inner region with negative D (see ref. 5) is emphasized here.

Solutions investigated in the case $S = 0$, $E = 0$, have subsonic flow ahead of the nucleus that is close to incompressible and possesses a weak bow shock^{5,6}. The shock is about Mach-2 at the nose and is very blunt, crossing $z = 0$ at some 2.5-3 times the stand-off distance; its Mach number increases towards the flanks⁷. Its strength is sufficient to diverge the flow ahead of any impenetrable inner region (magnetosphere/ionosphere), which would be smaller in size by an order-of-magnitude (for computational purposes, Biermann *et al.*⁷ assumed an artificially large inner cylinder, while Lipatov's^{8,9} magnetohydrodynamic calculation has an inner magnetic sheath conditioned by large gyroradius effects).

However, momentum and energy losses are likely to be very significant. Implanted cometary ions, entrained by electromagnetic fields associated with the plasma flow, gain much of the deceleration energy², having 40% of the total pressure behind the shock in the transverse field situation calculated by Galeev *et al.*¹⁰. When they undergo charge-exchanging reactions, such as



most of their energy and momentum is lost with the neutralized particle. This is sufficient to drive D [equation (1)] negative at low enough Mach number². When the ions are highly suprathermal, D can go negative at Mach numbers little less than unity¹⁰, as in a Venus interaction model².

An additional loss process for the energetic cometaries arises because they drift across the bulk plasma flow threaded by a magnetic field that is draped around the comet on the large scale^{11,12}. In such a field structure, fresh ions originating in the sunward coma generally diverge from the symmetry axis¹². Their mean effect is additional cooling and a loss of momentum that supplement the losses through charge exchange. The situation differs from that investigated by spacecraft at Venus or in the artificial AMPTE (Active Magnetospheric Particle Tracer Explorers) comet, where the obstacle and boundary layer scales were smaller than gyroradii of new ions. In such cases (see ref. 13), charge separation results in sheaths and streaming instabilities with intense, short-wavelength electric fields.

In the presence of strong cooling, Galeev *et al.*¹⁰ proposed a solution with re-accelerated flow and excluded any stagnation region. But that depends on the assumption of incompressibility:

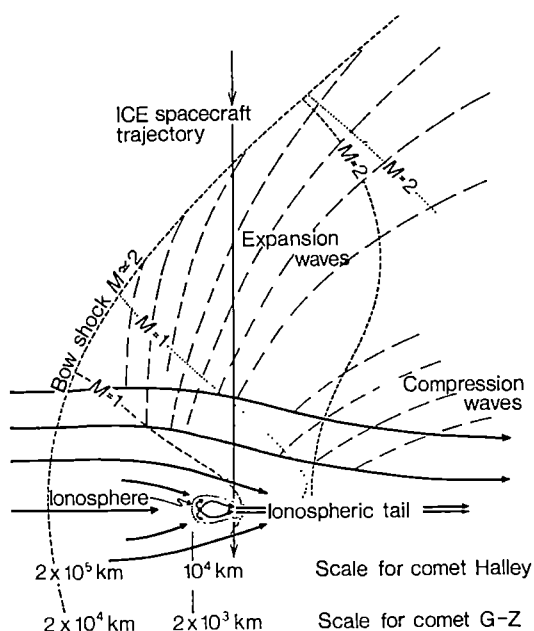


Fig. 1 Plasma flow through the coma under strong cooling. Schematic streamlines and expansion/compression wave patterns are sketched using the $M=1$, $M=2$ lines displaced upstream from those computed for mass-accreting flow (dotted lines).

$uA = \text{constant}$ which, combined with equation (2), requires $du = -Ddz/\rho > 0$. We choose the alternative behaviour, whereby streamtubes tend to reconverge due to low inner pressure: then $dA \approx 0$, which allows $du < 0$ [for $Ddz < 0$] continuing into the stagnating flow.

In this case, the flow pattern would be as sketched in Fig. 1. There is a small ionosphere plus tail of relatively cool plasma in the interior, with a stagnation region ahead but converging streamlines farther out. The sonic line is displaced sunwards from that computed in mass-source models⁷⁻⁹ and Mach numbers are higher behind the cooled coma, as indicated by the $M = \sqrt{(\rho u^2/\gamma p)} = 2$ line. The intermediate region of the coma acts more like a 'sink' than an ion 'source' due to its cooling power. The negative D acts to accelerate the supersonic flow². Qualitatively, we use gas-dynamic arguments^{4,14} to sketch patterns of expansion waves emitted from convex arcs of streamlines. Such expansion waves propagate out at the angle $\arcsin(1/M)$ to the flow and on reaching the flanks of the bow shock weaken it. They also bend the bow shock more sharply downstream. If the flow close to the ionosheath is primarily converging (as estimates indicate), the total weakening will be sufficient to cause complete decay of the shock's limbs, leaving Mach waves or propagating Alfvén waves. Of the two cases we discussed earlier¹⁵, the heliosphere with cooled, convergent wake is closest to the present case. The concave downstream streamlines emit compression waves, but the tailside coma provides decreasing friction ($S \uparrow 0$) like a supersonic diffuser, giving increasing Mach number in the emergent flow: the waves may, therefore, diverge as in Fig. 1, rather than converge to the classical tail shock. The innermost ionospheric tail can still, in principle, have the complex wave pattern of a supersonic jet¹⁵.

The gas-dynamic description of equation (2) needs modification to describe the magnetized flow properly. However, the differences are small when the field is weak, as is the case for energy density some 2–5% of the flow energy. Then, the magnetic field behaves primarily as though convected outside 0.1 times the shock scale¹¹. There is a magnetic contribution to the pressure with an effective γ of 2, which limits the potential compression effected by cooling. In addition, Maxwell stresses transfer some momentum from the solar plasma that is convecting the outer parts of draped field lines, to the cometary plasma that's dragging back the inner parts. The net result is extra acceleration of the sheath around the tail wake and ionosphere (Fig. 1) compared with the gas-dynamical case, but there is no qualitative difference

compared with our flow pattern.

The ICE spacecraft is targeted to fly through the wake of comet Giacobini-Zinner at $>10,000$ km downstream, that is at >0.3 times the shock stand-off scale¹⁶. It is hoped that the location of the shock flanks and the size of density and field jumps will reveal the nature of the shock and how far the solar wind "is deflected around or mingles with cometary plasma"¹⁷. We suggest, however, that interpretations will not be straightforward. The bow-shock flanks will be less widely flared and weaker than those given by the mass-source model and may be fully decayed. The main signature of the solar wind interaction will be the presence of energetic cometary 'pick-up' ions, whose places of origin will be complicated by significant drifts relative to solar protons.

The ICE spacecraft flew through the comet tail on 10 September at 8,000 km downstream. Initial results¹⁸ show an ionospheric tail and energetic ions over an extensive region but no bow shock, in agreement with our present prediction.

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Underwater noise caused by precipitation

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The characteristics of underwater noise in the ocean generated by precipitation are important to weather forecasters and oceanographers because they permit the detection and measurement of rain over the oceans by remote (buoyed or bottom-mounted) acoustic sensors. We have recently observed the characteristics of the underwater noise generated by rain, hail and snow. The spectrum of rain noise, for wind speeds below 1.5 m s^{-1} , shows a peak at 13.5 kHz with a sharp cutoff on the low-frequency side and a gradual fall-off (7 dB per octave) on the high-frequency side. Stronger winds smear the peak. Hail spectra show a peak at 3.0 kHz with a gradual (roughly 11 dB per octave) fall-off on both sides. The spectrum of snow noise is unique. Our instrumentation permitted the measurement of the drop (or stone) size distributions in the precipitation. These findings will enhance the art of remote acoustic sensing in oceanography.

Traditionally, the study of undersea acoustic noise has remained the province of the sonar designer and his co-workers because it sets limits on the detection capabilities of sonar equipment. The characteristics of such noise have been determined by many workers^{1–4} and the primary causes have been attributed to wind, shipping and, in shallow water, to biological organisms

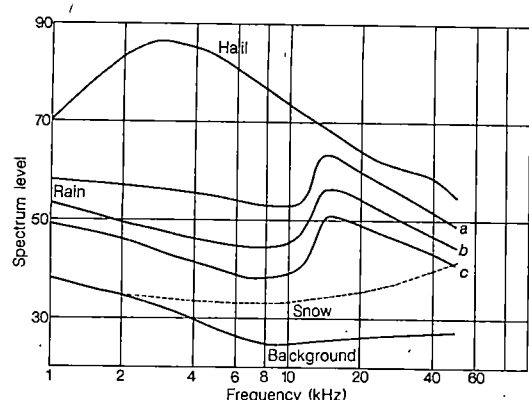


Fig. 1 Underwater noise spectra of rain (a-c), hail and snow for wind speeds $> 1.5 \text{ m s}^{-1}$. Spectrum level is expressed in dB relative to $1 \mu\text{Pa}^2 \text{ Hz}^{-1}$. Rain rates: a, 1.1 mm h^{-1} ; b, 0.29 mm h^{-1} ; c, 0.13 mm h^{-1} . Wind speeds: a, 2.7 m s^{-1} ; b, 2.7 m s^{-1} ; c, 2.23 m s^{-1} .

In recent years, 'the inverse problem' has been in vogue. Here the underwater noise becomes the signal, and measurement of this quantity in deep water appears to constitute a valid measurement of wind speed above the water surface. Measurements of wind speed and undersea noise level exhibit a high correlation, although relatively short-duration peaks in the noise records were attributed to rain by Shaw *et al.*⁵, who suggested the use of two or more observation frequencies to distinguish between wind- and rain-induced noise. Lemon *et al.*⁶ showed that undersea noise measured in three frequency bands centred at 4.3, 8.0 and 14.5 kHz on a continental shelf was readily inverted, through a simple logarithmic relationship, to wind speed within $\pm 1.5 \text{ m s}^{-1}$, for wind speeds in the range $0-12 \text{ m s}^{-1}$, and also obtained estimates, using Franz's⁷ data, of rainfall amounts which agreed within a factor of 2 with those obtained at nearby shore stations.

The undersea noise induced by precipitation has received little attention. Franz⁷ developed a theoretical expression for the spectrum of rain noise based on measurements of the underwater noise produced in a tank by sprays of droplets from a form of shower head. Heindsmann *et al.*⁸ reported some observations of undersea noise intensity resulting from rain showers in Long Island Sound, while Bom⁹ obtained spectra of underwater rain noise in the 1,200–9,600-Hz frequency band in Sarzana Lake in northern Italy. Nystuen¹⁰ has reported on some broadband measurements made in Clinton Lake.

During the winter of 1984–85, we made selected observations of underwater noise produced by carefully monitored precipitation in a roughly 4-km² arm at the eastern end of Cowichan Lake, South Vancouver Island, British Columbia, Canada ($48^\circ 48' 15'' \text{ N}$, $124^\circ 10' 12'' \text{ W}$). The forms of precipitation observed were rain, hail and snow, observations of the latter two forms being by chance rather than from experimental intent.

The hydrophone (an ITC 8084A having a near-spherical spatial response and a quasi-flat frequency response over the

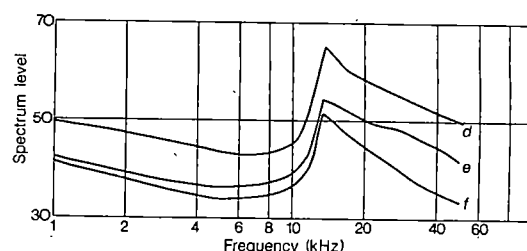


Fig. 2 Underwater noise spectra of rain for wind speeds $< 1.5 \text{ m s}^{-1}$. Spectrum level is expressed in dB relative to $1 \mu\text{Pa}^2 \text{ Hz}^{-1}$. Rain rates: d, 0.7 mm h^{-1} ; e, 0.2 mm h^{-1} ; f, 0.11 mm h^{-1} . Wind speeds: d, 0.76 m s^{-1} ; e, 0.59 m s^{-1} ; f, 0.45 m s^{-1} .

range 1–50 kHz) was mounted 0.7 m from the lake bottom in 35 m of water some 300 m from shore and its signal was carried by cable, after pre-amplification, to a recording facility ashore. After further amplification and improvement in the frequency response (flat $\pm 0.5 \text{ dB}$) over the frequency range, the signal was fed to an HP 3580A spectrum analyser using a 300-Hz-wide swept filter, which provided adequate resolution for the form of spectra observed and presented here. Wind speed and rain rate measurements were made ashore on a flat, bare tract of land virtually co-planar with the water surface, using conventional Government of Canada equipment and a Distromet RD69 distrometer.

Figure 1 shows underwater noise spectra due to hail, rain (a-c) and snow. The three rain noise spectra, corresponding to the rain rates given in Table 1, are typical of the bulk of the spectra for light rain which were collected. Figure 1 illustrates the characteristics of the rain spectra for wind speeds of $\sim 2.6 \text{ m s}^{-1}$ through a constant fall-off in sound level of about 7.0 dB per octave for frequencies increasing above 18 kHz, a rounded peak in the frequency interval 12–18 kHz and a rapid fall-off in sound level of roughly 45 dB per octave for frequencies decreasing below 12 kHz. Below 8 kHz, these spectra show more gradual slopes of $\sim 4 \text{ dB}$ reduction in level for each octave increase in frequency.

The noise spectrum produced by hail is quite different from that of the rain noise, showing a single broad peak centred at 3 kHz, with a fall-off of 10- and 12-dB per octave on the high- and low-frequency sides of the peak respectively. As one might expect, the noise spectrum due to snow is of low level, and is characterized by a gradual increase of the order of 3–5 dB per octave as the frequency increases.

Figure 2 shows a set of rain noise spectra taken in calm conditions. In contrast to those shown in Fig. 1, these spectra exhibit a very sharp cutoff at 13.5 kHz. The fall-off on the high-frequency side of the peak is between 6.5 and 10 dB per octave, essentially the same as those of the rain noise spectra taken with wind speeds of 2.6 m s^{-1} , while on the low-frequency side the spectral slope is 55–65 dB per octave. The low-frequency slope ($< 5 \text{ kHz}$) is 3 dB per octave, with the level decreasing

Table 1 Drop size distributions in terms of the number of raindrops (or hailstones) in a given diameter interval falling on 1 m^2 of the surface per s

Spectrum	Drop diameter interval (mm)															Total no. of drops ($\text{m}^{-2} \text{ s}^{-1}$)	Mean rain rate (mm h^{-1})	Kinetic energy (dB) re spectrum f
	0.3–0.4	0.4–0.5	0.5–0.6	0.6–0.7	0.7–0.8	0.8–1.0	1.0–1.2	1.2–1.4	1.4–1.6	1.6–1.8	1.8–2.1	2.1–2.4	2.4–2.7	2.7–3.0	3.0–3.3			
a	7	2	33	74	87	174	74	54	20	9	7	0	0	0	0	541	1.06	25
b	9	9	27	67	54	74	34	9	2	0	0	0	0	0	0	285	0.3	18.5
c	2	14	20	60	33	47	7	2	0	0	0	0	0	0	0	185	0.166	14.0
d	134	107	275	268	167	140	40	7	0	0	0	0	0	0	0	1,138	0.721	21.1
e	214	208	141	74	20	20	7	0	0	0	0	0	0	0	0	684	0.211	12.9
f	0	0	0	7	2	2	0	0	0	0	0	0	0	0	0	11	0.01	0
Hail	2	2	2	20	60	268	301	181	127	114	114	74	54	7	2	1,328		

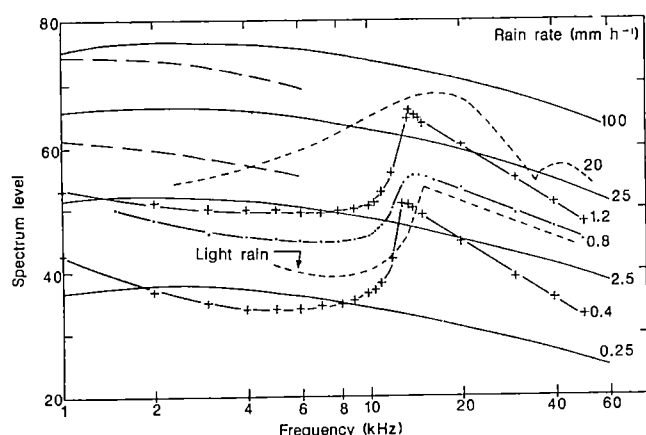


Fig. 3 Comparison of rain noise spectral samples due to Franz (—), Bom (—), Nystuen (---) and Scrimger (wind $< 1.5 \text{ m s}^{-1}$ (+—+), wind $> 1.5 \text{ m s}^{-1}$ (—)). Spectrum level is expressed in dB relative to $1 \mu\text{Pa}^2 \text{ Hz}^{-1}$. Numbers adjacent to curves are rain rate in mm h^{-1} .

with increasing frequency. Figure 3 compares our results with those reported by other workers.

The use of a distrometer permitted the determination of the drop size distributions in the rain producing the acoustic noise samples. These are given in Table 1, which shows the number of drops in a given drop-size interval, expressed in terms of the drop diameter, falling on 1 m^2 of the surface per s. Also given is the rain rate derived from the drop counts and the power input per m^2 at the surface due to the kinetic energy of the drops, expressed in dB, referred to the power associated with the rain noise of spectrum f . The latter was chosen as it was obtained from the lightest rainfall of the samples considered. The distrometer samples were 30 s long, so that three contiguous samples were added to correspond to a single 90-s sample obtained in near coincidence with the 100 s needed to produce the acoustic spectrum.

These data are remarkable for the abrupt fall-off in level of the rain noise spectra below 13 kHz and particularly for the sharp contrast in form between the spectra of the rain noise taken in winds of speeds $< 1.5 \text{ m s}^{-1}$ compared with those taken in winds of greater speed. In the 17 spectra obtained in winds of speeds below 1.5 m s^{-1} , which might well be considered 'pure' rain noise spectra, the peak occurs at 13.5 kHz within a few hundred hertz and the cutoff on the low-frequency side of the peak is extremely sharp, dropping the spectrum level over 15 dB in a 2-kHz frequency interval. Evidently, the wind, as it increases in speed above 1.5 m s^{-1} , affects the noise generation mechanism in such a way that the 13.5-kHz peak is rounded off and extended over several kHz and the slope on the low-frequency side of the cutoff is diminished. The hail spectrum above 15 kHz is essentially the same as the rain spectra but it does not exhibit the cutoff near 13.5 kHz, and peaks at 3.0 kHz. Considering the different properties of hail and rain—the rigidity of the hail and the fluidity and surface tension of the rain—the simplicity of the hail spectrum compared with that of the rain is not surprising. Also, *a priori*, the negative slopes of the respective spectra towards high frequencies are consistent with the concept that the higher-frequency energy in the noise is generated by the smaller drops (or stones) and that these input less energy (both kinetic and, in the case of rain, potential) at the water surface.

The noise spectrum of falling snow is unique in that it increases uniformly at 3 dB per octave as frequency increases over its entire range. As the kinetic energy of the falling snow is negligible compared with hail or rain, the noise must arise from a process associated with the melting of the snow.

I thank Mr Don Evans who designed the instrumentation for this experiment and assisted with the measurements and Messrs Wayman Yee, Vance Loiselle and Malcolm Scrimger who were involved in the equipment design and construction and also the data gathering. The work was supported by several agencies of

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Polymerization of silicate anions in solutions at low concentrations

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Adsorption of tetrahedral-forming ions, such as silicates and phosphates, is considered to be the main cause of retention of these anions on the surfaces of hydrated Al- and Fe-oxides. The importance of this retention in natural and man-caused phenomena has been the subject of many investigations. When adsorption studies are conducted, the role of the surface and the charges developed on it by the potentially determining ions tend to be emphasized, while the nature of the sorbate is rarely considered or investigated. Nevertheless, the excluded or equilibrium solution is the seminal source of information in the formulation of adsorption mechanisms or models. The ionic species present in the equilibrium solution are practically always taken for granted, that is, from data previously published. They are thought to be a simple function of solution pH, without further analysis of ionic speciation changes that occur during the sorption process. Here, we provide direct experimental evidence, using an advanced and sensitive spectroscopic technique, laser Raman spectroscopy (LRS), that aqueous equilibrium silicate solutions at low concentrations contain polymeric and/or other anionic complexes and not just monomeric species. These low concentrations are similar to those used in most previous sorption experiments on Fe- and Al-sesquioxides and on tropical acidic soils. Our findings clearly indicate that any mechanisms and models that are proposed to describe anion sorption on sesquioxides should consider that silicates, and probably some other tetrahedral-forming anions, are not present in solution solely as monomeric ionic species as most investigators have assumed^{1–11}. Irregularities in fitting experimental data to models previously reported prompted this research^{2–4,6,12}.

We determined that a spectrum of Na- or Ca-silicate could be obtained at very low Si concentrations and that the 'fingerprint' approach (Fig. 1) is applicable to the spectra obtained because they have lines distinctly placed, well separated, and of adequate intensity to determine the frequencies at which the peaks or bands occur. The published Raman spectra of Na silicates in solution were determined from Si solutions of concentrations 200–1,000 times larger than the solutions used in this study^{13–16}. No laser Raman (LR) spectra of Ca-silicates in solution have been published.

The spectrum of a Na-silicate solution at a Si concentration of $75 \mu\text{g cm}^{-3}$ is presented in Fig. 1. Numerous precautions were taken to exclude CO_2 from the alkaline solutions. Air-tight plastic bottles were used to store the silicate solution and all of the samples were freshly prepared just before the experiments with degassed water. The solution pHs were measured with a combination electrode immersed in a sealed plastic container to restrict any CO_2 contamination. Unless otherwise stated, all the solutions examined were adjusted to pH 10 since this pH is close to the reported pK of monosilicic acid, that is 9.5 (refs

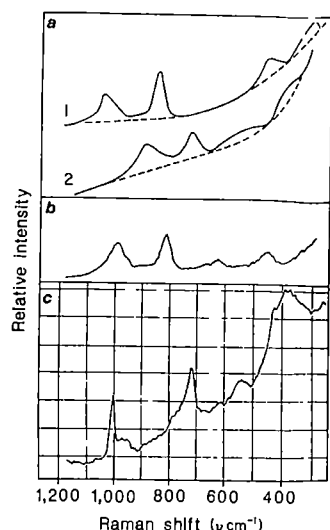


Fig. 1 Comparison of spectra of Na_2SiO_3 solutions reported by different authors. *a*, Raman spectrum as reported by Fortnum and Edwards¹³. 1, NaH_2PO_4 , 4 M solution (used as silicate analogue); 2, Na_2SiO_3 , 2.5 M. *b*, Laser Raman spectrum of Na_2SiO_3 1.5 M as reported by Freund^{14,15}. *c*, Laser Raman spectrum of Na_2SiO_3 (multipass device) ($\text{Si} = 75 \mu\text{g per g} \approx 0.003 \text{ M}$).

7–9). This pK_a is a crucial component of the conclusions of many previous investigations. As a basis for comparison, Raman spectra of 1.5 and 2.5 M silicate solutions previously reported^{13–15} are also shown. The remarkable intensity of the Raman lines for the low Si concentration solution is attributed to the enhancement of the signal due to the multipass device used in the studies. A very satisfactory spectrum may also be obtained with Si concentrations as low as $30 \mu\text{g Si cm}^{-3}$. More details of the methodology and the analogue structures suitable for these analyses will be published elsewhere.

Consideration of the position and number of the Raman shifts in the $400\text{--}500 \text{ cm}^{-1}$, 600 cm^{-1} , 785 cm^{-1} , and at the ≈ 900 and $1,050 \text{ cm}^{-1}$ regions agree fairly well. The slight displacement in the 900 and $1,000 \text{ cm}^{-1}$ regions may be due to pH differences. Solutions that are 1.5 or 2.5 M obviously have much higher pHs (> 12) than the $25\text{--}75 \mu\text{g cm}^{-3}$ working range of our Si solutions.

Calcium as well as Na salts are frequently supporting electrolytes in sorption studies, and soluble silicates occur at low concentrations in the soil solution, and in numerous other aqueous environments. When we examined solutions of low Si concentrations, 'anomalies' led to the suspicion that silicate species other than monomers were present. The indications of silicate polymerization or the formation of complexes other than monomeric silicates or silicic acid are reflected in the following LR spectral characteristics: (1) the band at $1,070\text{--}1,090 \text{ cm}^{-1}$ is considered to indicate the presence of a dimer (Si–O–Si band)^{13–15,17} or Si polymers^{13–16,17–20}; (2) changes in the band features, that is, splitting and/or broadening of peaks, shifts of the band frequencies to higher values, and changes of intensity (band height). The lines at $\approx 780 \text{ cm}^{-1}$ are especially sensitive to these changes^{14,15,17–19,21,22}.

The increase in polymerization of the silicate solution is reflected qualitatively by an increase in the intensity of the Raman lines at 780 and $1,070 \text{ cm}^{-1}$ (Fig. 2) in spectrum *b* compared with spectrum *a*. With increasing Si concentration, polymerization of Si rapidly occurs as shown by the development of the peak at $1,070 \text{ cm}^{-1}$ (refs 15, 16, 19–22).

Using times and ionic environments comparable to most other previous sorption studies^{1–6,12}, we determined that a silicate solution of low concentration is not only in monomeric form. Fresh monosilicic acid solutions ($75 \mu\text{g cm}^{-3}$) were prepared using the H-resin method⁸ and brought to pH 10 with NaOH and examined immediately by LRS (Fig. 3). Spectra were run at 15-min intervals and when the solution 'aged' at 3 h, distinct changes in the spectrum as appears in Fig. 3 inset *b* were clearly noticed.

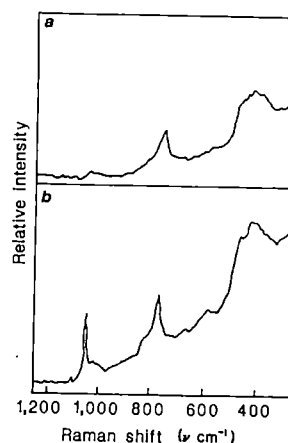


Fig. 2 Laser Raman spectra of aqueous solutions of Na_2SiO_3 with varying concentration. The increase in polymerization in graph *B* is reflected by an increment in the band $= 1,070 \text{ cm}^{-1}$, which corresponds to a silicate dimer (Si–O–Si band) or higher polymer. *a*, Na_2SiO_3 solution at $75 \mu\text{g cm}^{-3}$; *b*, Na_2SiO_3 solution at $100 \mu\text{g cm}^{-3}$.

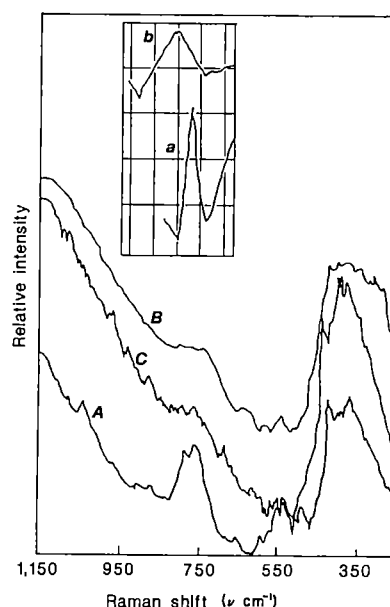


Fig. 3 Laser Raman spectra of Si-solutions at a Si concentration of $75 \mu\text{g per g}$. Ca(OH)_2 or Na(OH) were added to reach a $\text{pH} = 10$. This pH was selected because between $\text{pH } 8.5$ and 10.5 'maximum adsorption' or 'adsorption envelopes' are reported to occur. *A*, A freshly prepared solution of monosilicic acid and Ca(OH)_2 . *B*, Solution *A* examined after 'ageing' for 3 h after exposure to the laser beam. *C*, Solution *A* examined after ageing for 3 h with no previous exposure to the laser beam. No differences between *B* and *C* were detected, therefore the Si-solution was not polymerized by photocatalysis. Inset: *a*, A freshly prepared solution of monosilicic acid and NaOH. *b*, Solution *a* after ageing for 3 h. The $\approx 782 \text{ cm}^{-1}$ peak broadens, splits and shifts frequency as a sign of polymerization. The labelling of the axes are as in *A*.

The 782 cm^{-1} frequency observed (Fig. 3, insets *a* and *b*) was taken as a reference and it was shown from experimental evidence^{17,22} that the ≈ 780 peak is perturbed when Si polymerizes. This is evident because inspection of the spectra revealed three very distinct alterations of the aged solution in comparison with that at 'zero-time', (Fig. 3, insets *a* and *b*): (1) the band width broadened to twice the value at 'zero-time'; (2) the intensity (band height) decreased nearly 50%; (3) the frequency was displaced from 782 to 803 cm^{-1} . Effects of photocatalysis, due to the laser beam, on the rate of polymerization were ruled out by additional experiments in which Ca was the complementary cation (Fig. 3, *A*, *B*, *C*). Although not shown, a sample of $30 \mu\text{g cm}^{-3}$ Si was examined in similar conditions and even at

this concentration, variations of the 782 cm^{-1} peak occurred 3 h after sample preparation.

The present results are simply a predictable extension of proven facts which explain polymerization, anion bridging and/or gelation of inorganic polymers from solutions at higher concentration than in our studies^{8,9,21,23-28}. They indicate that it is difficult to define a boundary Si concentration, high or low, where silicate monomers are the sole species, which has been the repeated assumption in many previous investigations. Our results confirm that the formation of at least a silicate dimer, at pH 10, predicted from theoretical and experimental reported data^{23,28} occurs at any silicate concentration (in fact, 35% as silicate-dimer, Iler, unpublished data). Even at low concentra-

tions and after a short period of time, a substantial part of the silicate solution is non-monomeric. Moreover, the widely accepted concept that maximum adsorption of silicate on sesquioxides occurs at the $pK_a = \text{pH}$ of monosilicic acid^{1-6,12} without consideration that polymeric species exist has led to incorrect explanations and non-fitted models for those 'adsorption' systems. At $\text{pH} = pK_a$ the conditions are optimal for polymerization to occur^{25,26}. Indeed, our statements do not proscribe sorption of silicates on various sesquioxides in nature and in the laboratory^{1-6,12,22}. Work is in preparation which explains polymerization mechanisms at low concentration, and the possible configurations of dimers and polymeric species, based on evidence from spectroscopy and complementary techniques.

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Laboratory models for aromatization and isomerization of hydrocarbons in sedimentary basins

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With increasing depth in the Earth's crust, changes take place in the sedimentary distributions of aromatic steroid hydrocarbons and of stereoisomers of certain types of alkanes^{1,2}. The changes occur before and during the early stages of hydrocarbon generation and have been interpreted as resulting from aromatization and configurational isomerization reactions respectively³. We have now brought about an aromatization reaction and an isomerization reaction in the laboratory under free radical conditions, sulphur being chosen as a convenient source of radicals, and have extracted rate constants from the absolute concentration-time functions of the organic substrates. The activation energies and pre-exponential factors were then deduced from the temperature dependence of the rate coefficients. The values of these activation parameters show that, in the laboratory, the aromatization is more temperature dependent than the isomerization reaction, as suggested previously from the distributions of steroidal compounds in sedimentary basins³⁻⁵.

The relative extents to which the proposed reactions occur for steroid hydrocarbons in the sedimentary column have been suggested as being indicative of the thermal history of sediments³. These data, in conjunction with a geophysical model describing sedimentary basin formation, have been used to derive both activation energies and pre-exponential factors governing the rates of the reactions⁴⁻⁶. However, such studies have used kinetic schemes based on the following assumptions: (1) the reactions obey unimolecular first-order rate laws, and (2) the total concentration of reactant plus product remains constant throughout each reaction. In addition, rate coefficients have been derived from the relative abundances of presumed products to reactants, as measured from peak areas in mass chromatograms, rather than from absolute concentrations. The activation energy of a reaction is dependent upon its mechanism⁷; hence, if laboratory-derived values are ever to be extrapo-

lated to the sedimentary column, the same mechanism must be followed in both cases. Previous authors⁸⁻¹² have proposed the importance of free radical mechanisms in petroleum generation and associated processes. Significant concentrations of free radicals have also been measured, even at room temperature, in many kerogens¹³.

In the present study, intimate mixtures of the organic substrate, powdered sediment and elemental sulphur were heated at temperatures where high concentrations of sulphenyl and polysulphenyl radicals are observed¹⁴. Typically, the mixture in Pyrex glass tubes sealed *in vacuo* (after three N₂ purges) was heated in an oven and the temperature monitored using a chromel-alumel thermocouple. The substrate for aromatization was an isomeric mixture of ring-C monoaromatic hydrocarbons (compounds I, Fig. 1)¹⁵ and the matrix was a solvent-extracted carbonate sediment (Cretaceous, Abu Dhabi), with 0.08% w/w of elemental sulphur added. For the isomerization, one stereoisomer [(6*R*,10*S*) = *meso*-pristane] of 2,6,10,14-tetramethyl pentadecane (IIIa, Fig. 1) was used, with a solvent-extracted shale as matrix (Semécourt shale, Lower Toarcian,

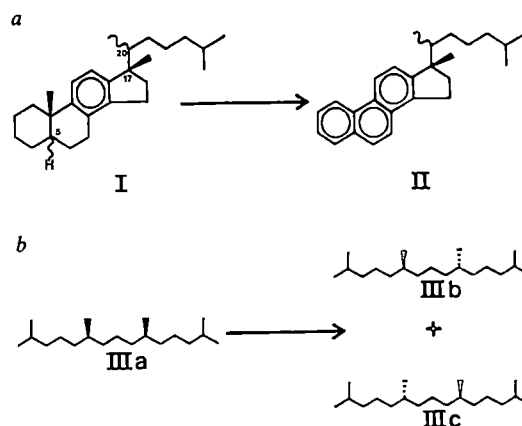


Fig. 1 a, Aromatization of the 5 α (H) and 5 β (H) isomers of (20*R*)- and (20*S*)-17 β -methyl-18-norcholesta-8,11,13-triene (I) to (20*R*)- and (20*S*)-17 β -methyl-18,19-dinorcholesta-1,3,5(10), 6,8,11,13-heptaene (II) (numbering based on steroid skeleton). b, Isomerization of (6*R*,10*S*)-pristane (IIIa) to (6*R*,10*R*)- plus (6*S*,10*S*)-pristane (IIIb and IIIc respectively).

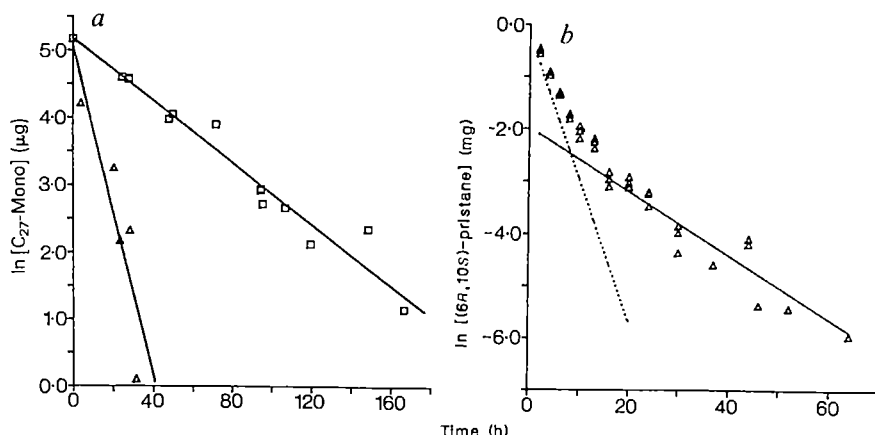
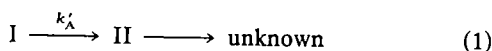


Fig. 2 a, A plot of $\ln[I](t)$ against heating time(h) where $[I](t)$ is the concentration of C_{27} ring-C monoaromatic steroid hydrocarbons. \square , 147.5 °C (420.5 K); Δ , 165 °C (438 K). b, Plot of $\ln[IIIa](t)$ against heating time (h) where $[IIIa](t)$ is the concentration of (6R,10S)-pristane at 250.2 °C (523.4 K).

Paris Basin) plus 10% w/w of elemental sulphur. After dichloromethane extraction, quantitative product analysis was achieved by the internal standards method, using capillary gas chromatography on OV-1 (or CP-Sil 5) and DEGS/PEGs (3/1) stationary phases¹⁵.

With increasing burial depth in sediments, an increase occurs in the relative abundance of triaromatic (base peak at m/z 231 in their mass spectra; for example compounds II) to monoaromatic steroid hydrocarbons (base peak m/z 253; for example I)^{2,16}. A similar change in the aromatic steroid distribution has been observed by heating a shale in the laboratory¹⁷. Furthermore, heating experiments similar to those described here have suggested the following reaction scheme¹⁵:



where k'_A = aromatization rate constant.

If the aromatization obeys a first-order rate law, then the predicted concentration-time function $[I](t)$ for the monoaromatic steroid is:

$$[I](t) = [I](t=0) \exp(-k'_A t) \quad (2)$$

Thus, if equation (2) is correct, a plot of $\ln[I](t)$ versus time will yield a straight line with slope k'_A and intercept $\ln([I](t=0))$. In the present study, there was an exponential decrease in monoaromatic concentration with increasing time and a concomitant increase in triaromatic concentration in the early time regime. Substitution in equation (2) of experimental data from two temperatures (147.5 ± 1.5 °C and 165.0 ± 1.0 °C) are plotted in Fig. 2a. Linear regression analysis gave close to straight-line fits with calculated intercepts 5.20 (147.5 °C) and 5.13 (165.0 °C). These match satisfactorily the actual value (5.17) from the initial concentration of monoaromatic steroids. The rate constants extracted from the slopes have values of 0.0228 h^{-1} (147.5 °C) and 0.1246 h^{-1} (165.0 °C). Straight-line fits were also obtained at two other temperatures (Table 1).

The temperature dependence of a rate constant may be used to deduce the activation parameters of a reaction by use of the Arrhenius expression:

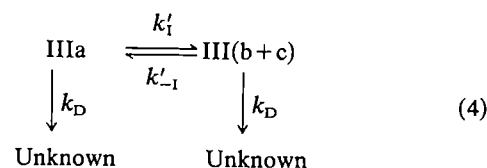
$$k = A \exp(-\Delta E_a/RT) \quad (3)$$

where R is the gas constant and T the absolute temperature.

The Arrhenius plot for k'_A , using data from Table 1, is shown in Fig. 3a. The slope yields an activation energy, ΔE_a , of 145 kJ mol^{-1} and a pre-exponential factor A , extracted from the intercept at $1/T = 0$ of $\sim 6.7 \times 10^{12} \text{ s}^{-1}$.

In immature sediments, pristane occurs predominantly as the (6R,10S)-diastereoisomer (IIIa); with increasing thermal stress, a 1:1 mixture of the (6R,10S) to the (6R,10R) plus (6S,10S) isomers is eventually formed (IIIa to IIIb plus IIIc)^{1,18}. The same effect has been observed when a shale is heated in the laboratory¹⁷. In the present work no isomerization was observed when the pure (6R,10S)-substrate was heated alone in a medium of liquid sulphur. When heated on the shale matrix with sulphur, absolute quantification reveals that, in parallel with isomeriz-

ation at C-6 and C-10, there is a degradative process depleting the total amount of pristane, as proposed previously¹⁵. These observations are consistent with the following reaction scheme:



The rate constants for the two side reactions are assumed to be equal because of the diastereomeric relationship of the product to the precursor in this acyclic alkane. Additionally, rate constants for the forward and reverse isomerization are assumed to be equal because a 1:1 equilibrium mixture of precursor and product is formed. Analysis of scheme (4), with the assumption of first-order kinetics, predicts that the concentration-time function for (6R,10S)-pristane should behave as follows:

$$[IIIa](t) = a_1 \exp(-\lambda_1 t) + a_2 \exp(-\lambda_2 t) \quad (5)$$

where

$$\lambda_{1,2} = k'_1 + k_D \pm k'_1 \quad (6)$$

The $\lambda_{1,2}$ are the roots of the auxiliary equation:

$$\lambda^2 - 2(k'_1 + k_D)\lambda + k_D(2k'_1 + k_D) = 0 \quad (7)$$

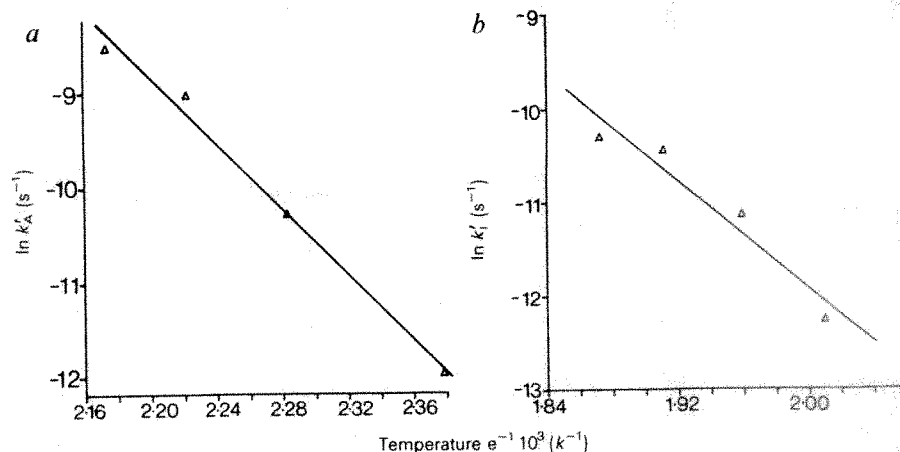
Equations (5) to (7) predict that the reactant concentration-time function should consist of two decreasing exponentials. Figure 2b shows that the experimental data fit this prediction. The solid line from linear regression analysis of points corresponding to $t > 21$ h, represents the longer-lived component ($\lambda_2 = k_D$). When this line is extrapolated to $t = 0$ and subtracted from the data points at early times ($t < 21$ h), a regression line (dashed) through the resulting residuals yields the short-lived component which has the time constant $\lambda_1 = 2k'_1 + k_D$. Thus, k'_1 at 250.2 °C is 0.1063 h^{-1} (Fig. 2b). Results for this and three other tem-

Table 1 Aromatization and isomerization rate constants as a function of temperature

Aromatization	
Temperature (K)*	$k'_A (\text{h}^{-1})^\dagger$
420.5 \pm 1.5	0.0228 \pm 0.0015
438.0 \pm 1.0	0.1246 \pm 0.0270
450.0 \pm 1.0	0.4418 \pm 0.0186
460.0 \pm 1.0	0.7206 \pm 0.0575
Isomerization	
	$k'_1 (\text{h}^{-1})^\dagger$
497.4 \pm 1.5	0.0173 \pm 0.0015
510.4 \pm 1.3	0.0534 \pm 0.0079
523.4 \pm 2.3	0.1063 \pm 0.0080
534.2 \pm 1.9	0.1230 \pm 0.0540

* \pm Maximum observed range.

† Error = ± 1 s.d.

Fig. 3 Arrhenius plots for: a, k'_A and b, k'_I .

peratures are collated in Table 1. Using expression (3) and the data in Table 1, the activation parameters were extracted from the Arrhenius plot (Fig. 3b); ΔE_a has a value of 120 kJ mol^{-1} and A is $\sim 2.1 \times 10^7 \text{ s}^{-1}$.

The laboratory heating experiments show that a steroid aromatization reaction to give the m/z 231 triaromatics and a configurational isomerization reaction can be brought about in the laboratory under free radical conditions. Although first-order kinetics appear to be followed in each case, it is likely that pseudo-first-order rate constants have been measured since there is evidence that the overall rates of reaction are dependent on sulphur concentration¹⁵.

It is difficult to know at present whether these preliminary laboratory experiments, using single compound types, represent an appropriate model for the increase in extent of aromatization of aromatic steroid hydrocarbons and in the number of isomers in certain types of alkanes observed in sediments with increasing thermal stress. On the other hand, if the aromatization and isomerization reactions do indeed occur in sediments, and if free radical mechanisms operate, the laboratory ΔE_a values may be applicable to the sediment reactions. It must be recognized, however, that the apparent A factors, measured in the laboratory, are unlikely to be applicable to sediments; they are probably enhanced in the laboratory as a result of the increased concentration of radical initiator¹⁹. Despite these complexities and the fact that the laboratory ΔE_a values differ from those derived in a semi-empirical manner, using the geophysical model⁴⁻⁶, it is noteworthy that the Arrhenius plots (Fig. 3) show that the aromatization is the more temperature-dependent reaction, that is, it has larger ΔE_a (145 kJ mol^{-1}) and A ($6.7 \times 10^{12} \text{ s}^{-1}$) values than the isomerization reaction (120 kJ mol^{-1} and $2.1 \times 10^7 \text{ s}^{-1}$ respectively). Similar behaviour is implied by the relative extents of aromatization of aromatic steroid hydrocarbons and of isomerization in the side chain of steroid alkanes in that aromatization is accelerated relative to isomerization in sediments with more rapid heating rates³⁻⁵.

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Palaeoclimatic and tectonic implications of Neogene microflora from the Northwestern Ethiopian highlands

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Climatic changes in East Africa have been well documented although the record is far from complete. Palaeoclimatic data for both the Holocene and Pleistocene¹⁻⁷ and the Pliocene¹⁻⁸ are available through fossil pollen studies. However, these data extend in a continuous manner only as far back as 3.7 Myr BP (ref. 11) largely because of the emphasis on studies related to human origins. Fragmentary data from macrofossils also exist for older periods from Eastern¹⁴⁻²⁰, Central and Southern Africa^{21,22} although the scarcity of material and the lack of isotopic dates makes it difficult to define continuity in the patterns of climatic fluctuations in East Africa. The late Miocene pollen/spore flora described here represents the first data from East Africa for this time period and provides new information on the pre-Pliocene flora and climate of the continent. The pollen flora comes from a post-8-Myr lacustrine deposit in the heart of the Northwestern Ethiopian Plateau ($12^{\circ} 35' \text{ N}$, $37^{\circ} 06' \text{ E}$) and contains 'exotic' taxa that are now extinct. The pollen diagram is characterized by abundant wet lowland rainforest taxa and pteridophytes, a very weak representation of grasses and the total absence of conifers, indicating warm and humid climates towards the close of the Miocene. Post-depositional uplift of the Chilga area ($\sim 1,000 \text{ m}$) is also implied from the fossil pollen/spore assemblage.

The Chilga fluvio-lacustrine, lignite-bearing sedimentary sequence occurs within a graben, 60 km south-west of Gondar

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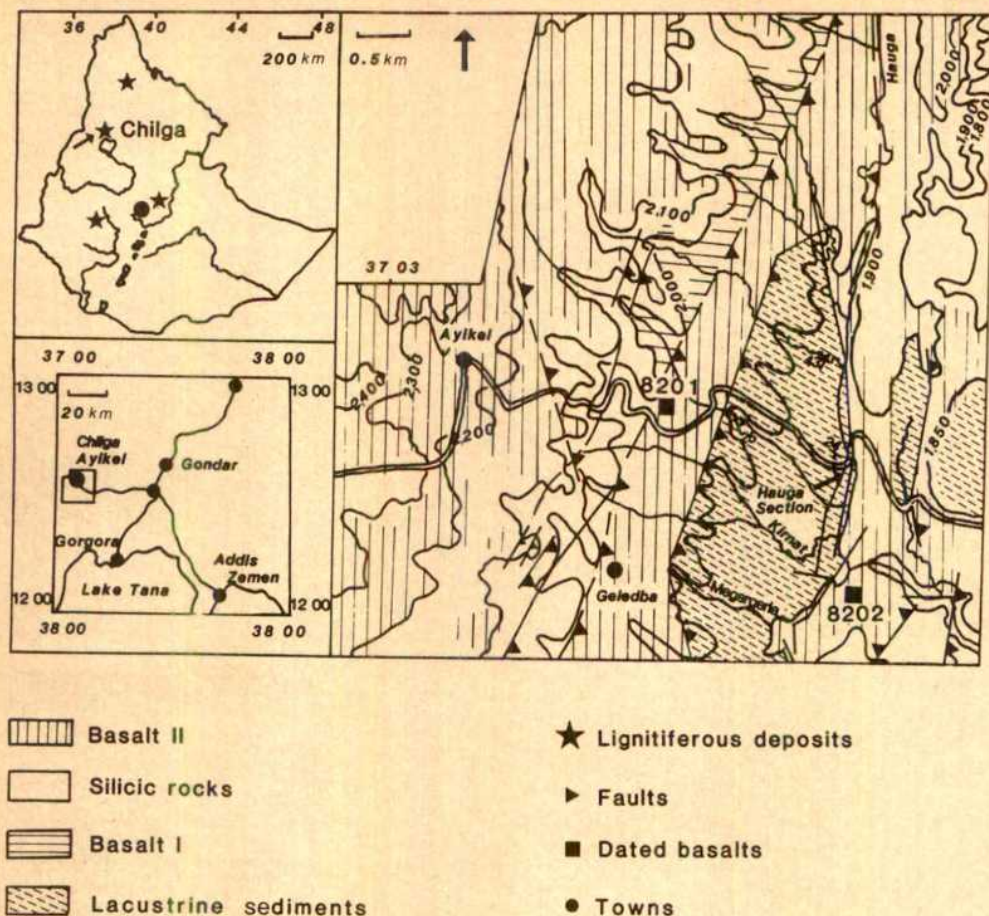


Fig. 1 Location map of Chilga and the sections studied (geological map after Tezera and Heeman²⁶).

and ~100 km north-west of Lake Tana, at altitudes between 1,900 and 2,000 m (Fig. 1). The graben is part of a large defunct Eocene rift, which ceased activity 25–20 Myr ago as the transverse-trending Afar proto-rift began to form²³. The sediments occur in one of the small north-south (NS) grabens [13 km NS by 2 km EW (east-west)] within the major Tana graben, formed by the NNE-SSW fault line running parallel to the Ethiopian Rift system. The Chilga graben is bounded on the west by highlands which rise abruptly to higher than 2,300 m. The formation of these grabens is attributed to renewed faulting activity associated with late Tertiary volcanism²⁴.

The Chilga sedimentary sequence is one of the ubiquitous continental sedimentary intercalations within the Oligo-Miocene Trap Series on the Plateau. The sequence directly overlies a basalt layer (Basalt II) which forms the graben floor. This basalt also covers the top of the westward-lying highlands and consists of numerous lava flows from separate eruptive episodes^{23,26}. It is represented by stratified, fine-grained olivine basalt (KY8201) on the highlands and is recognized along the fault line in the Chilga basin by its amygdaloidal leucite pockets and calcite veins (KY8202). Potassium-argon whole-rock analysis of two rock samples from Basalt II gave ages of 9.8 ± 0.5 and 8 ± 1.2 Myr respectively (collaboration with I. Villa, University of Pisa). The sedimentary sequence (34 m thick) commences with medium-grained basal conglomerates which fine upward to coarse-grained sandstones. It then abruptly changes to black carbonaceous mudstones which are intercalated with very fine lignite seams and thick silt layers. In the lower part, silts, clayey silts and clays dominate the section, whereas thick and consolidated lignite beds frequently occur between +15 and +30 m. A 3-m-thick water-lain volcanic ash layer crowns the sedimentary sequence.

The climate in Ethiopia is controlled by macro-scale pressure changes and monsoon flows²⁷, with elevation and topography contributing variation on the Plateau²⁸. The Northwestern Plateau is under a regime of summer rain with a mean annual rainfall of 1,000–1,400 mm distributed in one single season with

a dry period of 5–7 months²⁹. A local maximum of 1,500–1,800 mm on the mountain east of Lake Tana and 950–1,500 mm around the lake is observed¹⁸. The warmest season (25 °C) is March–May; the mean temperature on the Plateau is 5 °C cooler.

The vegetation of the Northwestern Plateau belongs to the Afromontane archipelago-like regional centre of endemism³⁰. Further south, an island of undifferentiated woodland vegetation dominated by Combretaceae associated with *Balanites aegyptica*, *Piliostigma thonningii* and *Gardenia ternifolia* form a halo around Lake Tana. Remains of evergreen montane forest with *Olea africana*, *Podocarpus gracilior*, *Juniperus procera* and *Sapotaceae* occur above 2,000 m on the well-studied east side of the Lake Tana region^{32,32}. Stands of natural vegetation have now disappeared under the pressure of human settlement³³.

Forty-six complete pollen spectra were obtained from a total of 96 studied samples collected from two outcrop exposures within the Chilga basin. Vitrinite-rich lignites and coarse detrital sediments preserved no fossils, whereas intertinite-rich lignites, carbonaceous mudstones, clays and silts yielded rich pollen and spore assemblages. Identification of fossil pollen grains was achieved with the help of a reference collection of 6,000 modern species of Ethiopian and East African flora^{34,35} and the available literature on pollen morphology^{36,37}.

We recorded 121 identified and 2 unidentified pollen taxa, with only 0.5% of unknowns on the total pollen/spore count. The Chilga fossil pollen assemblage is unique in Africa in its floral composition and diversity. This fossil flora was extensively compared with post-Eocene macrofossils from the north-west, central and south-west parts of the Ethiopian plateau^{15,18,19}, Kenya¹⁴ and Uganda²⁰; with palynoflora from central, south-east and north-east Ethiopia^{38,39}, Burundi²¹ and South Africa²²; with the modern Ethiopian flora^{35,40} and with the neighbouring East African flora⁴¹. The comparisons show three striking features in the composition of the post-8-Myr flora from Chilga, namely, the presence of 'exotic' taxa, the absence of *Podocarpus* and *Juniperus*, and the abundance of pteridophytes.

The fossil flora contains taxa that are foreign to the modern,

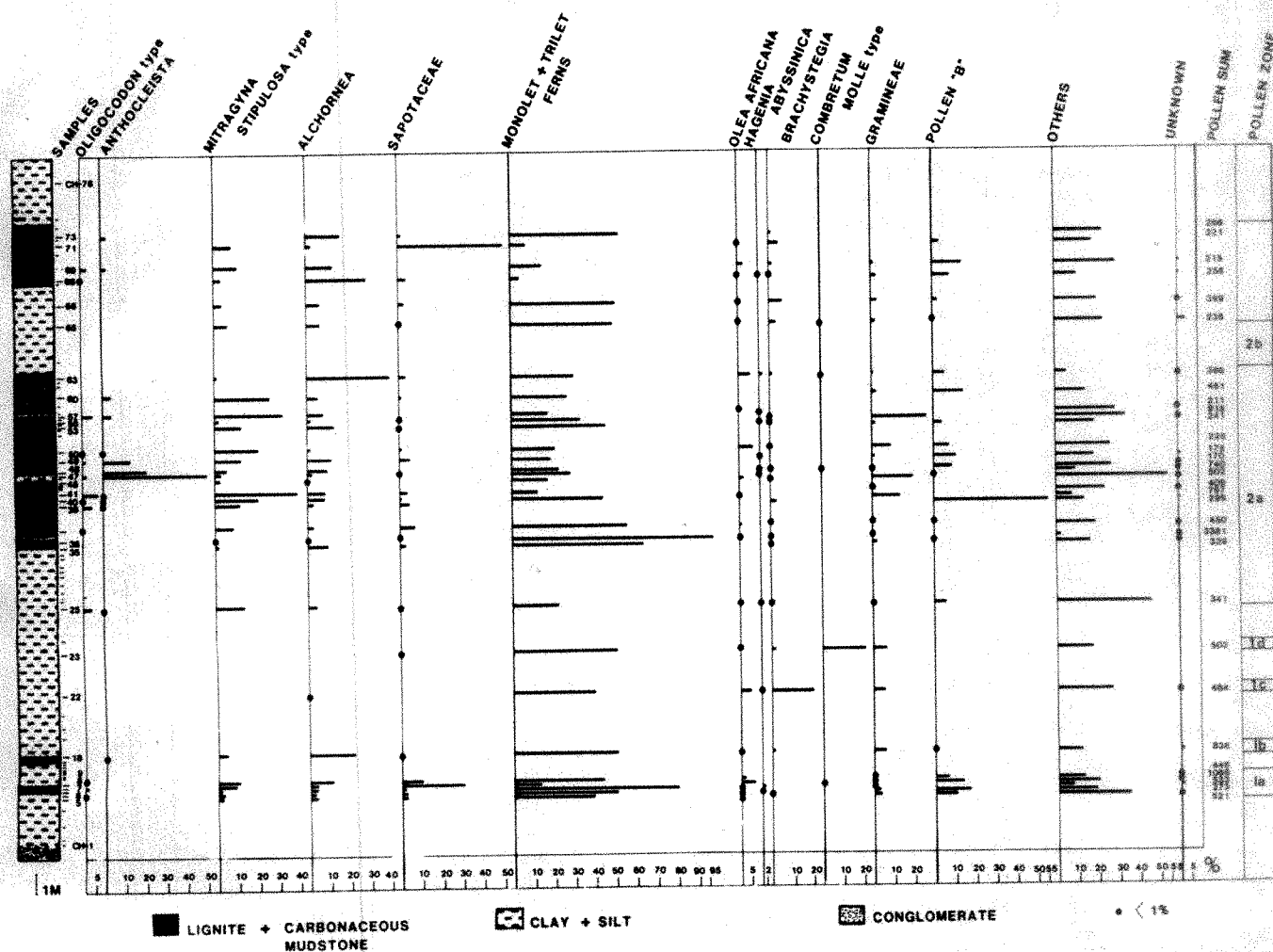


Fig. 2 Pollen diagram of the Chilga lacustrine deposit; relative frequencies calculated with respect to the total pollen/spore count.

Pleistocene and Pliocene pollen flora from East Africa³⁹. These include *Afrocrania volkensii*, *Brachystegia*, *Isobertlinia* type, *Iodes* type, *Holoptelea grandis* type, *Oligocodon* type and *Rauvolfia*. Although *Afrocrania* does not exist in the present Ethiopian flora⁴⁰, it is a major element of wet montane vegetations up to 3,200 m and down to lake water edges in moist montane forests in East Africa. The nearest *Brachystegia* and *Isobertlinia* vegetations are found today >1,000 km south of Chilga, in northern Tanzania, with a southward arm extending into Zimbabwe⁴¹. *Oligocodon*, a climbing shrub in tropical West Africa, has so far been recorded only from the wet rainforests of Nigeria and Cameroun³⁷. On the other hand, a taxon with pollen type indistinguishable from *Oligocodon*, *Gardenia imperialis*, grows along stream, lake and swamp margins in Uganda and Congo. *Holoptelea* is a widespread species in the Guineo-Congolian phytogeographical region as an important component of semi-evergreen lowland rainforests³⁰. The pollen of *Rauvolfia vomitoria* recorded from the rainforests of West Africa³⁷ has similar pollen types: *Rauvolfia caffra* and *Rauvolfia manni*, species which occur in montane forest vegetation in Burundi and Tanzania. The presence of these 'exotic' taxa in association with the abundant lowland taxa such as *Anthocleista*, *Mitragyna* and *Alchornea* strongly suggests a closed forest environment that is far more humid than present-day forests in the highlands of Ethiopia.

Podocarpus gracilior is a species that is a widespread component of upland forests throughout Ethiopia and East Africa. *Podocarpus* is found in East Africa in forests where the mean annual rainfall is within the range 800–1,600 mm at altitudes between 1,800 and 3,000 m. On the Western Plateau, it occurs between 1,500 and 2,200 m in nearly pure stands. *Juniperus procera*, the other associated conifer, is abundant in dry montane forests, at altitudes above 1,700 m and is also a major component

of the forest zone between 2,900 and 3,000 m altitude. The absence of a single pollen grain of either of these two conifers in a total pollen/spore count of 24,452, specially considering the excellent export ability of *Podocarpus*³, is solid evidence for the absence, on the regional scale, of present-day type upland forests on the plateau during the period of deposition. The absence of conifers is also one factor which physiognomically differentiates Guineo-Congolian rainforests from Afromontane rainforests³⁰.

More than seven different types of spores were observed in the Chilga pollen/spore flora. Except in the Upper Neogene palynoflora from Burundi²¹, which has an even more important spore flora, the Plio-Pleistocene palynoflora from East Africa does not show such diversified and abundant ferns as the Chilga pollen/spore assemblage. Elevated frequencies of pteridophytes denote an increase in rainfall and runoff⁴², thus suggesting that the Chilga fossil flora may represent the moist and shady conditions of a closed, wet forest.

Percentage frequencies of the pollen and spore assemblage are shown in Fig. 2 (detailed manuscript in preparation). This pollen diagram is condensed from the larger and more complete Hauga section (31 spectra). It comprises only taxa characterized by their high pollen concentration, consistent presence and/or generic significance. Three important features distinguish Chilga from other East African Tertiary sites.

(1) Dominance of arboreal taxa: the arboreal taxa make up >50% of most spectra (>70% if pteridophytes are excluded), a distribution pertaining to the structure of closed forests. No pollen diagram from the East African Plio-Pleistocene shows such high arboreal frequencies.

(2) Weak representation of grasses: most pollen diagrams from East Africa are characterized by high frequencies of *Gramineae* which often account for >50% of the total pollen count^{8,11,13}.

indicating widespread savanna and open woodland vegetation in the Plio-Pleistocene. In the diagram from Ghilga, the Gramineae frequencies, where present, are low, 0–27% (maximum in the moist pollen zone IIa), and the frequencies of the sedge (mostly absent) do not reach 15% (not included in this diagram). This also suggests a closed forest environment. (3) Instability of the palaeovegetation: six distinct development phases are recorded in the palaeovegetation during the depositional period. The earliest *Mitragyna* swamp forest developed extensively with *Alchornea* (Ib) at the expense of a montane-like vegetation (Ia). Through an unrecorded transition, it gave way to a deciduous *Brachystegia* forest (Ic) which further developed into a drier phase of *Combretum-Lepidagathis* woodland (Id). This vegetation was then completely replaced by an *Anthocleista-Mitragyna-Oligocodon* association of a fringing swamp and wet lowland forest (IIa). Towards the end of the period of deposition, a moist secondary forest with an *Araliaceae-Alchornea-Sapotaceae* association (IIb) became dominant, with *Sapotaceae* comprising >55% of the assemblage in the uppermost spectrum. These frequent changes from a closed swamp forest to semi-deciduous forest reflect a decrease in the humidity on the plateau.

The rate of uplift for the high plateaus of Ethiopia is thought to have increased exponentially since 100 Myr ago, from <0.05 mm yr⁻¹ to 1 mm yr⁻¹ at present⁴³, bringing the Jurassic marine limestone to its present altitude of 2,500 m (ref. 44). Epeirogenic uplift in the Cenozoic occurred at ~0.1 mm yr⁻¹ (ref. 45). If we assume a uniform uplift rate of 0.1 mm yr⁻¹, the Chilga lake beds may have risen 800 m during the past 8 Myr, which tentatively places the Chilga region at an altitude of around 1,000 m during deposition. This is also supported by the presence or absence of certain 'elevation-indicator' plant taxa in the palaeofloral assemblage. *Podocarpus* has previously been used to indicate palaeoaltitudes and to infer uplift on the Western Plateau⁴⁶. The absence of both *Podocarpus* and *Juniperus* from the Chilga palaeoflora, the modern presence of these conifers in the highlands and the location of the Chilga lacustrine deposit at an elevation of around 1,900 m suggest initial depositional altitudes of around 1,000 m for the Chilga area. Active episodes of regional tectonic and volcanic activity are also recorded from the distribution of clay minerals (<2 µm) in the sedimentary sequence which shows an abrupt increase of illite and smectite towards the top of the lake beds.

One little known but important aspect of the late Cenozoic vegetation history of Africa is the extension of tropical rainforests and the timing of the establishment of the savanna. Savanna as well as lowland rainforests and montane vegetation are thought to have occupied tropical Africa since the Miocene⁴⁶. The contraction and later disappearance of the eastern Tethys Sea during the Miocene initiated the fragmentation of the closed forest. Faunal evidence indicates a shift from lowland to woodland vegetation in tropical Africa by the mid-Miocene⁴⁷. The woodland vegetation was further fragmented by the occurrence of a widespread savanna-mosaic during the late Miocene/early Pliocene⁴⁸. Significant contraction of the tropical lowland rainforests and establishment of the savanna appears to fall within the mid-Miocene. On the Western Ethiopian Plateau, macrofossils of montane forest taxa have been recorded from several sites^{16,17}. However, no fossil record documenting lowland rainforest is available, although there exists an isolated patch of lowland rainforest³⁰ about 400 km south of Chilga. The present study, however, provides evidence for the presence of a closed forest with strong Guineo-Congolian affinities as late as 8 Myr BP as far north as 12° N on the Northwestern Ethiopian Plateau. Furthermore, the lack of datable material at the top of the sedimentary sequence allows one to speculate on a link between the late Miocene global climatic deterioration and the disappearance of the humid post-8-Myr Chilga flora. The flora may either represent the humid climate and vegetation of the Plateau before the terminal Miocene salinity crisis at 6.5–5.5 Myr BP or a relict vegetation which survived the Mediterranean aridity. Whatever the case may be, a change towards a cooler

and more arid climate in the circum-Mediterranean region that has been related to global Messinian events⁴⁹, should have noticeably affected the climate of the high plateaus of Ethiopia.

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Archaeobacterial lipids in hot-spring microbial mats

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Modern and ancient sediments contain a multiplicity of organic compounds which record a partial history of the organisms that contributed them¹. Interpretation of this record depends on the knowledge of links between the molecular markers and their biological, notably microbial, sources. This goal is made difficult

Table 1 Isoprenyl ethers (in μg per g dry weight)* of hot-spring microbial mats in Yellowstone National Park

Spring	Temperature (°C)	pH	SO ₄ ²⁻ (mM)	Archaeobacterial content† and activity	Phytanyl	Biphytanyl				Total	Average cyclization‡
						Acyclic (0)	Monocyclic (1)	Bicyclic (2)	Tricyclic (3)		
Octopus Spring (0–15 mm)	55	7.2–9.4‡	0.16	Active methogenesis/ numerous methanogens (10^7 – 10^8 cells cm^{-3})	17.2	8.5	2.3	1.5	—	12.3	0.43
Octopus Spring	70	8	0.16	Less active methanogen- esis/fewer methanogens ($\sim 10^2$ cells cm^{-3})	0.4	0.3	0.3	0.2	—	0.8	0.88
Painted Pool	37	6.5	7	Methanogenesis suppressed by sulphate reduction/few methanogens ($< 10^2$ cells cm^{-3})	0.3	—	—	—	—	—	—
Nymph Creek	47	2.8	ND	Thermoacidophiles likely	0.2	0.2	0.2	0.6	<0.1	1.0	1.4

—, Not detected. ND, Not determined.

* Samples of 52–8,000 mg dry weight were extracted and yielded of the order of 6 mg or less of purified alkanes derived from ethers. Peaks were identified by comparison to reference mass spectra (see ref. 17) and by spectral analysis. Peak areas were determined using a V.G. Analytical Minichrom Data System, and were corrected to concentration using response factors determined by replicate injections of standards (n -C₁₈ for phytane and n -C₂₈ for biphytanes). Coefficients of variation were 0.16–0.21.

† Methane production studied by following increases in methane during anaerobic incubation of mat samples. Estimations of methanogen population density studied by most-probable-number determination. See refs 19, 20 and 22 for details.

‡ pH determined by microelectrode (see ref. 27).

§ (% monocyclic + 2 × % bicyclic + 3 × % tricyclic) × 10⁻² after ref. 21.

|| Formulae given in Fig. 1.

by the complexity of modern sediment communities and the general lack of studies which directly tackle the relationships between the microbial inhabitants of natural systems and their associated organic compounds. We now report the first direct comparison between isoprenyl ether-linked lipids in hot-spring microbial mats and the observed populations of archaeobacteria. The particular mats examined are from Yellowstone National Park and may represent modern analogues of Precambrian communities preserved as stromatolites^{2,3}. As relatively simple microbial systems, they are suitable for validating specific molecular markers as indicators of microbial populations within a natural community. We find that the phytanyl and biphytanyl ethers reflect the measured distribution of methanogenic archaeobacteria.

An understanding of the relationships between a given biological species and its characteristic molecular markers relies on the analysis of appropriate cultures and, where possible, natural collections of that species. For many different types of land plants and algae, such procedures are relatively straightforward. For example, the link between the occurrence of 4-methylsteroids in sediments and dinoflagellates suggested by numerous sterol analyses of cultured dinoflagellates has been clearly established through direct comparison of the 4-methylsteroids of a collection taken from the water column with those of underlying sediments⁴. Similar approaches can be adopted for bacteria. For example, the occurrence of several specific acyclic isoprenoids among the free lipids of archaeobacteria⁵, a newly defined group of procaryotic microorganisms^{6–8}, grown in the laboratory was taken to suggest that they were characteristic of such organisms. Further circumstantial evidence in support of this proposition stemmed from the occurrence of these isoprenoid alkanes in sedimentary environments from which methanogenic archaeobacteria had been isolated⁹.

In addition to their free lipids, axenic cultures of representatives of the major archaeobacterial groups possess uniquely characteristic isoprenoid ethers. For example, halobacteria contain phytanyl (C₂₀)⁵ and sesterterpanyl (C₂₅)¹⁰ ethers, whereas methanogenic and other archaeobacteria (thermoacidophiles and other related organisms, such as *Thermoproteales*¹¹) produce a mixture of phytanyl and biphytanyl (C₄₀)⁵ ethers. Also, thermoacidophilic archaeobacteria can be distinguished from methanogenic archaeobacteria in that they alone are known to synthesize cyclic biphytanyl lipids⁵. All of these acyclic and cyclic isoprenoid ethers have been identified in modern and ancient sedimentary environments and fossil fuels¹² through chemical analysis. Such investigations have led to the suggestion that cyclic biphytanyl ethers are not restricted to thermoacidophiles, but may occur in methanogens¹³. An alternate interpretation is that other nonmethanogenic archaeobac-

teria are (or were) unknown inhabitants of sediments in which these cyclic biphytanyl ethers are found.

Clearly, a better understanding of the link between molecular markers and their microbial sources is desirable. For microorganisms, the traditional approach to the enrichment and isolation of axenic cultures does not ensure recovery of all the members of natural communities. Thus, the extent to which existing collections of axenic cultures (and their molecular markers) are representative of the total variety of organisms (and organic chemicals) in a given environment is not known. Lipids have been used as biochemical markers to assess directly the microbial community structure of sediments^{14,15}, but the complexity of sediment communities can seriously limit attempts to establish firm and direct relationships between organisms and their marker lipids. We have, therefore, begun investigations of such relationships in relatively simple systems, that is, hot-spring microbial mats, where high temperatures and/or acidity limit the diversity of their wholly microbial communities. However, microbial processes that control elemental cycles in these mats are similar, in principle, to those found in other aquatic systems¹⁶. These mats are also modern analogues² of the abundant Precambrian microbial communities recorded as stromatolites in the sedimentary record³. The significance of stromatolites stems from their status as a primary manifestation of life on Earth and, thus, they have a key role in the understanding of early evolution. The simplicity of the hot spring community reduces the problems of defining the relationships between organisms and molecular marker compounds. Yet, these mats are relevant analogues to the interpretation of microbial community structure in modern and ancient sediments and mats.

Samples were collected from microbial mats in three springs of Yellowstone National Park, Wyoming, USA (Table 1), using solvent-rinsed materials, immediately frozen, and lyophilized. They were treated directly with BBr₃ to release ether linked lipids as bromides, which were reduced to alkanes or D-labelled alkanes using LiAlH₄ or LiAlD₄, respectively¹⁷. Alkanes were analysed quantitatively by glass capillary gas chromatography and identified by computerized gas chromatography-mass spectrometry (C-GC-MS)¹⁸ by comparison with reference mass spectra for biphytane and its monocyclic analogue¹⁷, or by spectral interpretation. The degradative procedures released isoprenoid hydrocarbons in all samples (Table 1). The D-labelling suggested that these hydrocarbons originated from phytanyl monoethers and biphytanyl diethers.

Octopus Spring is an alkaline spring low in sulphate in which methanogenesis is active^{19,20}. Here, the highest concentrations of archaeobacterial lipids were observed in the 55 °C mat (Table 1) with phytanyl and biphytanyl ethers in approximately equal

Table 2 Depth distribution of isoprenyl ethers (in μg per g dry weight)* in Octopus Spring mat

Depth interval (mm)	Phytanyl	Biphytanyl			Total	Average Cyclization*
		Acyclic† (0)	Monocyclic (1)	Bicyclic (2)		
0-3	—	0.5	0.3	0.2	1.0	0.70
3-6	26.1	15.3	3.8	3.9	23.0	0.41
6-9	24.8	16.5	4.8	2.0	23.3	0.38
9-12	32.6	9.0	2.3	1.0	12.3	0.35
12-15	2.5	1.1	0.5	0.4	2.0	0.65

—, Not detected.

* See Table 1.

† Formulae given in Fig. 1.

abundance. This lipid distribution resembles that of *Methanobacterium thermoautotrophicum*⁵, the only methanogenic bacterium as yet isolated from this mat²⁰. The concentrations of these compounds within the mat were similar to the distribution of methanogenic bacteria and methanogenesis in the mat. They were higher at 55 °C than at 70 °C (Table 1), where methanogenesis is slight and methanogens are orders of magnitude less numerous^{19,20}.

The vertical distribution of isoprenyl ethers in the 55 °C Octopus Spring mat reveals that the highest concentrations occur in the 3-6 and 6-9 mm depth intervals (Table 2). This profile is consistent with that of methanogenesis which is most active in the 1-5 mm depth interval¹⁹. Our current method of analysis cannot, however, distinguish the complex polar lipids of living methanogenic bacteria from their degradation products retaining the isoprenyl cores. Hence, the ether cleavage products at depth in the mat may derive, in part, from the relict lipids of inactive methanogens, since methanogenesis does not occur below about 5 mm in the mat. Certainly, the isoprenoid glyceryl ethers survive in ancient sediments and even in pre-troleum¹².

Cyclic biphytanyl ethers were detected in the Octopus Spring mat (Fig. 1a), although they have not been reported in *M. thermoautotrophicum* or other methanogenic bacteria. Rather, they are representative of thermoacidophilic and related archaeobacteria, but such organisms have not been isolated from mats of alkaline springs. The unexpected presence of cyclic biphytanyl ethers in this mat parallels that of the discovery of cyclic biphytanyl ethers in marine sediments and fossil fuels^{12,17}. It may be due to either lipid inputs from other archaeobacteria, or a lack of complete understanding about methanogen lipids. The simplicity of the hot-spring mat community should permit a test of the first hypothesis, and we are now developing methods to do so. The higher degree of cyclization in biphytanyl ethers observed at 70 °C than at 55 °C (Table 1) may be due either to different archaeobacterial populations, or to the influence of temperature on the lipids of a single organism. For example, *Calderiella acidophila*, a thermoacidophilic archaeobacterium, shows greater cyclization of biphytanyl ethers with increasing growth temperature²¹. Cyclization also varied with depth, but no specific trend with depth was noted.

Painted Pool is a neutral spring high in sulphate. Although methanogenic bacteria may be enriched from such high sulphate environments^{22,23}, they exist at low population density and their activity is suppressed by sulphate reduction²². These environmental features are mirrored in the low concentration of phytanyl ethers and absence of biphytanyl ethers (Table 1). Similar results have been reported for methanogen lipids in lacustrine compared to marine sediments¹⁵, where methanogenesis is limited by the level of sulphate available in the sediment²⁴.

Nymph Creek is an acid spring of 47 °C which might harbour thermoacidophilic archaeobacteria²⁵. Here, cyclic biphytanyl ethers dominated over their acyclic counterpart (Table 1; Fig. 1b), with bicyclic components the most abundant. Tricyclic biphytanyl ethers were only detected in low concentration (Table 1). Similar features have been reported for thermoacidophilic

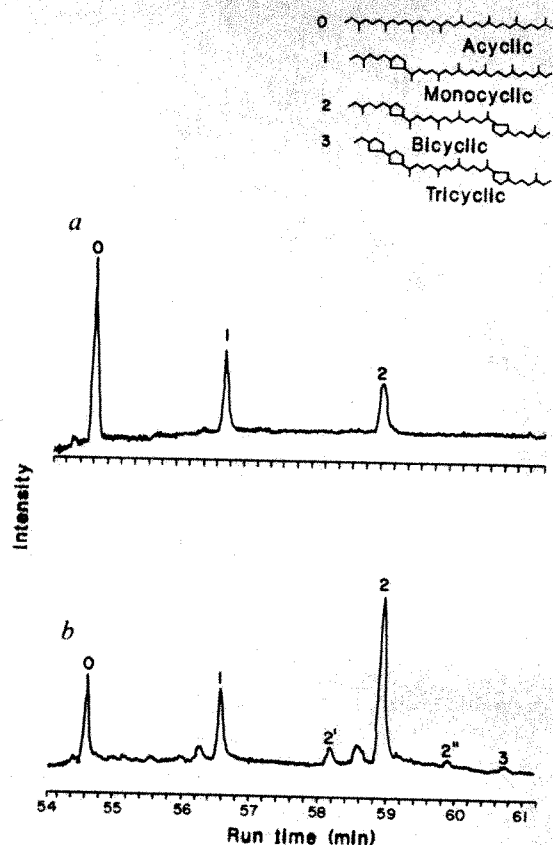


Fig. 1 Glass capillary gas chromatograms of alkanes derived from the ether cleavage procedure from: *a*, Octopus Spring 55 °C mat, 12-15 mm depth; and *b*, Nymph Creek 47 °C mat, ~0-1 cm depth. Samples were analysed on a Carlo Erba model 4160 gas chromatograph with a 50 m CPSil5CB WCOT column with H_2 carrier gas. Samples were injected on column at ~35 °C, and temperature was rapidly increased to 80 °C, then programmed to increase at 4 °C min^{-1} to 300 °C. Oven temperature was then kept isothermal at 300 °C (beginning at a run time of 55 min). Output from the flame ionization detector was processed on a V.G. Analytical Minichrom data system. Individual peaks were quantified by reference to the peak area responses of standard *n*-alkanes. *n*-alkanes were removed by urea adduction²⁶ before analysis of the sample shown in *a*. 2', 2'' = isomers of bicyclic biphytane¹².

archaeobacteria³, but despite numerous attempts, such organisms, especially *Thermoplasma*, have not been isolated from this mat²⁵. The only other known thermoacidophilic archaeobacterium which might inhabit this mat is *Sulfolobus*, but apparently it cannot be enriched from acid environments at temperatures as low as that of this mat²⁵. Other related archaeobacteria (*Thermoproteales*) would not be expected¹¹ to grow at this temperature and pH. Thus, the observation of archaeobacterial lipids in this hot acid mat illustrates our incomplete understanding of the microbial inhabitants of this system and suggests the presence of archaeobacteria which have thus far eluded enrichment and isolation.

This geochemical study of a well characterized environment provides evidence for the correspondence between the distributions of specific molecular markers for methanogens and the organisms themselves. Such assessments of the organisms contributing to a given environment using molecular markers are inevitably limited by the available information on the biological occurrences of the compounds. However, where diagnostic markers are found they can provide direct evidence of the activity of specific microorganisms. In modern environments, diagnostic molecular markers may reveal the presence of otherwise unknown community members. By contrast, the absence of such molecular markers cannot always be taken as indicative of a lack of contributions from the relevant organism, since biological and chemical alterations of organic compounds may occur

after biosynthesis, but before analysis. In addition, it is inappropriate to argue that molecular markers provide a quantitative assessment of the importance of individual organisms as sources of organic matter in general, especially when the compounds are present at levels <100 p.p.m. (parts per 10⁶).

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Complementary DNA sequences of ovarian follicular fluid inhibin show precursor structure and homology with transforming growth factor- β

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Inhibin, a specific and potent polypeptide inhibitor of the secretion of follicle-stimulating hormone (FSH)¹, of gonadal origin and thus a potential contraceptive, may constitute a missing link in the mechanism controlling the differential secretion of the pituitary gonadotropins. Inhibin-like bioactivity has been reported in various fluids and extracts of testis²⁻⁵ and in ovarian follicular fluid⁶⁻¹⁰. Although there have been several attempts to purify inhibin from

seminal plasma¹¹⁻¹³, purification from follicular fluid has been more successful (refs 14-16; for review see ref. 17). We have previously isolated two forms (A and B) of inhibin from porcine follicular fluid¹⁴. Each form comprised two dissimilar subunits of relative molecular mass (M_r) 18,000 (18K, referred to here as the α -subunit) and 14K (the β -subunit), crosslinked by one or more disulphide bridge(s). Forms A and B differ in the N-terminal sequence of their 14K subunit. Preliminary structural characterization of porcine¹⁵ and bovine¹⁶ ovarian inhibins shows that they have similar properties. Here, we have used the N-terminal amino-acid sequence data on the subunits of each inhibin to identify cloned complementary DNAs encoding the biosynthetic precursors and report that inhibins are the product of a gene family that also includes transforming growth factor- β (TGF- β) and whose structural organization is similar to that of pituitary and placental glycoprotein hormones.

The strategy for identifying clones containing coding sequences for the inhibin subunits was based on the 'long-probe' approach^{18,19}. A high-complexity cDNA library constructed in the vector λ gt10 and derived from porcine ovarian messenger RNA by oligo(dT)-primed cDNA synthesis was screened with a single 64-base synthetic oligodeoxynucleotide directed against the N-terminal amino-acid sequence of the porcine inhibin α -chain. Sequence analysis of several hybridizing cloned cDNAs confirmed correct probe identification and revealed that none of the cDNAs characterized encoded the complete structure of the α -chain precursor protein.

A second library was constructed by 3' extension on ovarian mRNA of a synthetic oligodeoxynucleotide complementary to a sequenced region encoding α precursor residues 60-64 (Fig. 1a). This library was screened with a suitable restriction fragment from a previously analysed cDNA and yielded several isolates which specified the remainder of the DNA sequences encoding the N-terminal region of the α precursor. The full sequences for the precursor protein and its cDNA are shown in Fig. 1b. The complete protein consists of 364 amino acids ($M_r \sim 39K$), of which the C-terminal 134 constitute the α -subunit. The pair of arginines preceding these 134 residues probably constitutes the site for proteolytic release of the α -subunit from its precursor. The pro-region of this precursor contains additional arginine pairs, suggesting the possible existence of other bioactive peptides within this precursor. The precursor protein sequence predicts two N-linked glycosylation sites, one within the α -chain proper. Glycosylation could account for the difference in size between that deduced from the amino-acid sequence ($M_r \sim 14.5K$) and the M_r of 18K obtained by gel electrophoresis¹⁴.

The cDNA sequences encoding the precursors of the inhibin β -subunits were obtained from the same cDNA libraries as used for the α -subunit. Overlapping cDNA clones were isolated by screening first with single long synthetic oligodeoxynucleotide probes based on the two N-terminal β -subunit sequences and subsequently with suitable restriction fragments derived from characterized cDNA clones which served as probes for 'walking' in both 5' and 3' directions (Fig. 2a).

Only four out of 2×10^5 clones from the oligo(dT)-primed library hybridized with the synthetic probe for the β -subunit of inhibin A. Three of these clones proved correct by DNA sequence analysis, but none contained a full 3'-untranslated region, as judged by the absence of a poly(A) homopolymer at their 3' ends. Unexpectedly, a higher abundance (~ 10 -fold) of inhibin β_A -subunit coding sequences was found in the cDNA library made by specific priming on α -subunit mRNA. This library was screened with the synthetic probe for the β -chain of inhibin A on our subsequently refuted working hypothesis that the α precursor mRNA might also encode the β -subunit. The high abundance of inhibin β_A cDNA in this library was later traced to a fortuitous complementarity of the specific α -chain primer to a region in the 3'-untranslated part of the β_A mRNA.

Only four cloned cDNAs encoding the β -subunit of inhibin B were found in our libraries. The sequence information

a

λ PIN- α 5A
 λ PIN- α 5
 λ PIN- α 4s
 λ PIN- α 10
 λ PIN- α 2
 λ PIN- α 9
 λ PIN- α 12s

5' 0 250 500 750 1000 1250 AAA 3'

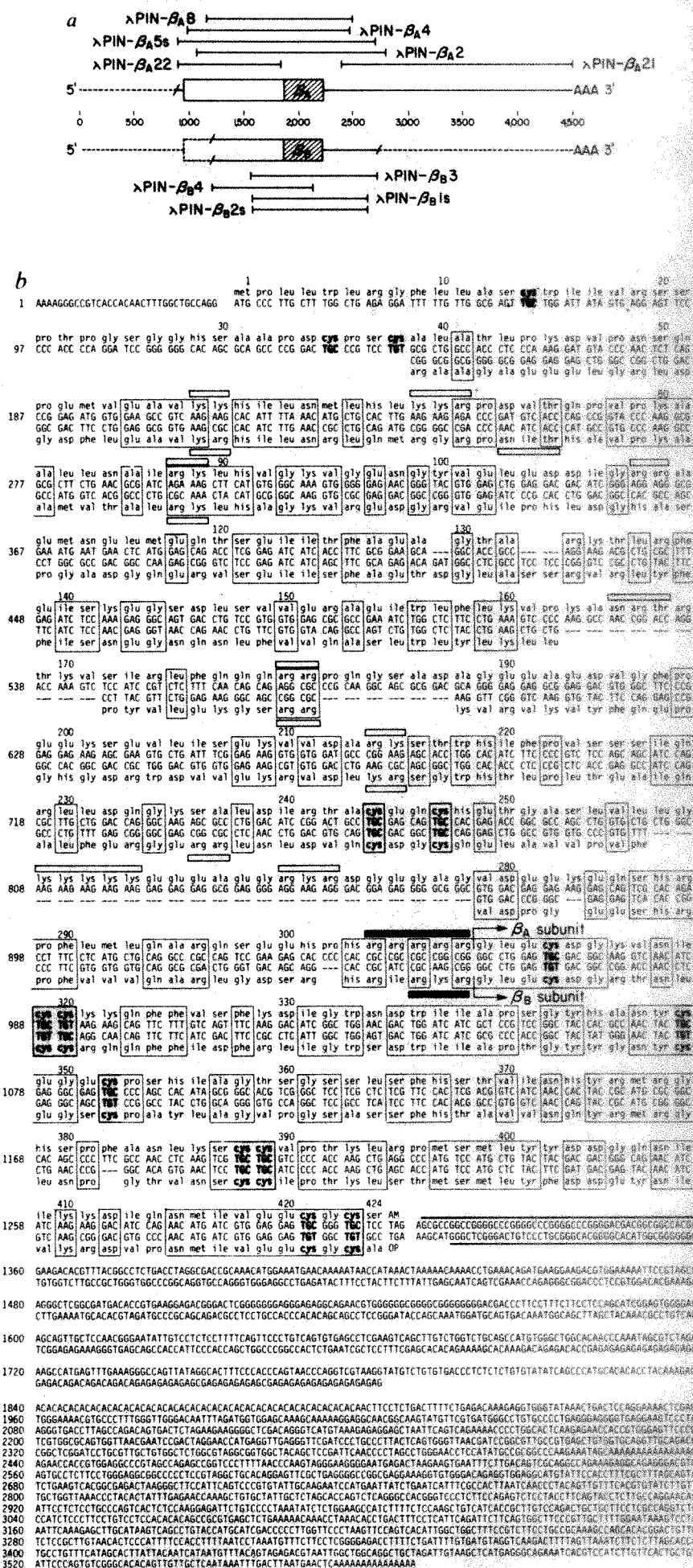
b

1 TGTGGGGCAGACCTTGACAGAAAGGGGACAGGGCTGGGTGTGGTTTCCCGTTGGCAGGGCCAGGTGAGCT 10 met trp pro gln leu leu leu leu leu ala pro
CGG AGT GGG CAT GGC TGC CAG GGC GCG GAG CTG GAC CCG GAG CTT GTC CTG GCC AAG GTG AGG GCT CTG TTC CTG GAT GCG TTG GGA CCC
arg ser gly his gly gln gln pro glu leu asp arg glu leu val leu ala lys val arg ala leu phe leu asp ala leu gly pro
CGG AGT GGG CAT GGC TGC CAG GGC GCG GAG CTG GAC CCG GAG CTT GTC CTG GCC AAG GTG AGG GCT CTG TTC CTG GAT GCG TTG GGA CCC
pro ala val thr gly glu gly gln asp pro gly val arg arg leu pro arg arg his ala val gly gly phe met arg arg gly ser glu
CCG GCA GTG ACT GGG GAA GGT GCG GAT CCT GGA CTG AGG GGT CTG CCC CGA AGA CAT GCT GTG GGG GGC TTC ATG CCG AGG GGC TCT GAG
pro glu glu glu asp val ser gln ala ile leu phe pro ala thr gly ala arg gln gln asp glu pro ala ala gly glu leu ala arg
CCC GAG GAG GAG GAT GTC TCC CAG GCC ATC CTT TTC CCG GCT ACA GGT GCC CCG TGT GGG GAG GAC CCA GCT GCT GGA GAG CTG GCC CGG
glu ala glu glu gly leu phe thr tyr val phe arg pro ser gln his thr his ser arg gln val thr ser ala gln leu thr phe his
GAG GCT GAG GAG GGC CTC TTC ACA TAT GTA TTC CCG CCG TCC CAG CAC ACA CAC AGC CCG CAG CTG ACT TCA GCT CAG CTG TGG TTC CAC
thr gly leu asp arg gln gly met ala ala asn ser ser gly pro leu leu asp leu leu ala leu ser ser arg gly pro val ala
ACG GGA CTG GAC ACA GAG GGG ATG GCA GCC GCC AAT AGC TCT GGG CCC CTG CTG GAC CTG CTG GCA CTA TCA TCC AGG GGT CCT GTG GCT
val pro met ser leu gly gln ala pro pro arg trp ala val leu his leu ala ala ser ala leu pro leu leu thr his pro val leu
GTG CCC ATG TCA CTG GGC CAG GCG CCC CCT CCG TGG GCT GTG CTG CAC CTG GCC CCC TCT GCC CTC CCT TTG TTG ACC CAC CCA GTC CTG
val leu leu leu arg gln pro leu gln ser ala arg pro glu ala thr pro phe leu val ala his thr arg ala arg pro pro
GTG CTG CTG CTG CCG TGT CTT CTT TCT TCC TCA CCG CCG CCC GAG GCC ACC CCC TTC CTG GTG GCC CAC ACT CCG GCG AGC CCA CCC
 α subunit
ser gly gly glu arg ala arg ser thr ala pro leu pro trp pro trp ser pro ala ala leu arg leu leu gln arg pro pro glu
AGC GGA GGG GAG GAG GCC CGA CGC TCC ACC GCC CCT CTG CCC TGG CCT TGG TCC CCC GCC CCG CTG CAG CTG CTG GAG ACC CCG GAG
glu ala ala val his ala asp gln his arg ala ser leu asn ile ser phe gln glu leu gly trp asp arg trp ile val his pro pro
GAA CCC GCT GTG CAC GCC CAG TGC CAC AGA GCT TCC TCC AAC ATC TCC TTC CAG GAG CTG GCG TGG GAC CCG TGG ATG CTG CAC CCT CCC
ser phe ile phe his tyr gln his gly gly gln leu pro thr leu pro asn leu pro leu ser val pro gly ala pro pro thr pro
AGT TTC ATC TTC CAC TAC TGT CAC GGG GGC TGC GGG CTG CCG ACC CTG CCC AAC CTG CCG CTG TCT GTC CCT GGG GCC CCC CCT ACC CCT
val gln pro leu leu leu val pro gly ala gln pro gln gln ala ala leu pro gly thr met arg ser leu arg val arg thr thr ser
GTC CAG CCC CTG TTG TTG GTG CCA GGG GCT CAG CCG TCC TCC GCT GCT CTC CCG GGG ACC ATG AGG TCC CTA CCG GTT CCG ACC ACC TCG
asp gly gly tyr ser phe lys trp glu thr val pro asn leu leu thr gln his ala leu leu OC
GAT GGA GGT TAC TCT TTC AAG TAC GAG ACG GTG CCG AAC CTT CTC ACC CAG CAC TGC GCC ATC TAA GGGGTGCCGTGGTGGCCGAGCTCCC
ACAGCCACCAAGCCTGAGGACAGGAGTCCCACTCCCTTCTCTTCGCTCTCCGCTGGAGGCTCCCTCCCTCTCGCCGCTTCCCAATGGTAATGTGCAATAAACAGCAT
AGTGACAGTGAATCGGTGCGCAAAAAAAA

Ovarian total and polyadenylated RNAs were analysed by the Northern procedure to assess size and relative abundance of the mRNAs encoding the peptide subunits of both forms of inhibin. DNA sequences encoding the divergent pro-regions of β_A and β_B were used as hybridization probes in stringent conditions. Analysis showed (Fig. 3) that α and β mRNAs are of different size and abundance, confirming results obtained from

Fig. 2 Inhibin β -subunits; nucleotide sequence of cDNAs and predicted primary structure of precursors. **a**, Schematic representation of the porcine β_A and β_B -subunit mRNAs with coding sequences boxed. The β_A and β_B -subunits (cross-hatched boxes) are encoded towards the 3' end of the coding sequences. The 3'- and 5'-untranslated regions are shown as a line. The length of the 5' end of β_A mRNA and the 5'- and 3'-untranslated regions of the β_B -subunit mRNA are inferred from the size of the mRNAs (Fig. 3) and the obvious similarity of β_B to β_A mRNA. Regions of the cDNAs not obtained by cloning are shown as dashes in the diagram. The relative positions of the overlapping oligo(dT)-primed cDNA clones and the randomly primed clones (λ PIN- β_A 5s, λ PIN- β_B 1s and λ PIN- β_B 2s) are indicated. The scale is in nucleotides from the 5' end of the 4.5-kb mRNA. **b**, Sequence comparisons. Nucleotide numbers on the left refer to those of cloned β_A cDNA sequences. Amino-acid numbers are for β_A precursor residues. The N-termini of the β -subunits, which are preceded by basic processing sites (black bars), are indicated by arrows. The β_B sequence is shown underneath the β_A sequence and aligned with it for maximum homology. Regions containing identical amino-acid sequences are boxed. Cysteine residues are shaded, possible processing sites are indicated by open bars, and potential glycosylation sites are shown by cross-hatched bars. A highly G+C-rich region 3' to the termination codon is underlined in one and overlined in the other sequence, and the AATAAA polyadenylation signal is underlined in the β_A -subunit sequence. There was one nucleotide difference between λ PIN- β_A 8 and the other clones covering this area: a G \rightarrow A change at nucleotide 868 results in a change of amino-acid 278 from a glycine to a serine. Another polymorphism was observed at amino-acid 206 in the β_B -subunit sequence shown, where the T \rightarrow C change found only in λ PIN- β_B 1 would result in a Phe \rightarrow Leu change.

Methods. Approximately 2×10^5 oligo(dT)-primed ovarian cDNA clones were screened with the 5'-³²P-labelled β_A oligonucleotide, 5'-AAGAAGCAGTTC-TTTGTGTCCTCAAGGACATTGGCTGGA-ATGACTGATCATTCG-3', based on the amino-acid sequence (residues 321-339). Four hybridization positives were obtained, of which three proved to contain β_A coding sequences (λ PIN- β_A 2, β_A 4, β_A 8). A 5'-end 157-bp *Eco*RI/*Hind*III (nucleotides 158-298) fragment and a 3'-end 221-bp *Eco*RI/*Pst* fragment (nucleotides 1,681-1,885) derived from λ PIN β_A 2 were used to screen 2×10^6 oligo(dT)-primed cDNA clones and 2×10^5 clones from the specifically α -chain primed library. Of the 16 clones analysed in detail, 2 were found to have longer 5' ends (λ PIN- β_A 5s, β_A 22) and 1 (λ PIN- β_A 21) contained the entire 3'-untranslated region. The *Eco*RI restriction sites do not refer to sequences present in the cDNA but are all contained within the cDNA adaptor fragment and thus add an extra 16 nucleotides to the above fragment sizes. Porcine inhibin β_B -subunit cDNA clones were isolated by screening 2×10^5 clones from the specifically primed library with the β_B oligonucleotide, 5'-GGCTGGAGTGTGATGGGAGACCAACCTGTCTCCGCCAGCAGTTC-TTCATCGATTTCAGGCT-3', which was based on the NH₂-terminal sequence described in Fig. 1a legend. Positive clones were further screened with the oligonucleotide inosine probe 5'-AAITCTAIAIA-ACTG¹TG-3' containing deoxyinosine³⁵, which covers all the possibilities in the noncoding strand for the amino-acid sequence QOFFIDE. Two clones (λ PIN- β_B 1s, β_B 2s) were sequenced and found to code for the β_B -subunit. A 231-bp *Eco*RI/*Sma* (nucleotides 406-630) fragment was isolated from λ PIN β_B -1 and used as a hybridization probe to screen 2×10^6 oligo(dT)-primed cDNA clones. Two positives were obtained (λ PIN- β_B 3, β_B 4). The nucleotide sequence of these overlapping clones was used to construct the sequence shown. All sequences were obtained by subcloning specific fragments into M13 phage vectors^{33,34}.



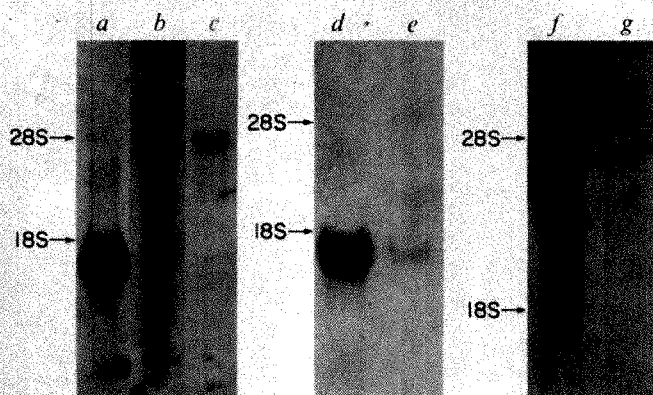


Fig. 3 Northern analysis of mRNA for inhibin subunits α , β_A and β_B . Lanes *a*, *b*, *c*, *d* and *f* contain poly(A)⁺ RNA and lanes *e* and *g* contain total RNA. The positions of the 28S and 18S ribosomal RNAs are indicated by arrows. Lanes *a*, *d* and *e* were hybridized with an α -subunit cDNA probe, lanes *b*, *f* and *g* with a β_A -subunit-specific probe, lane *c* with a β_B -subunit-specific probe. The α -subunit mRNA is ~1.5 kb long, the β_A -subunit mRNAs are ~4.5 and 7.2 kb, and the β_B -subunit mRNA is ~4.5 kb long. The hybridizations shown in lanes *a*, *b* and *c* were performed with probes of approximately equal length and specific activity in order to judge relative mRNA levels.

Methods. Polyadenylated RNA (2 μ g, lanes *a*, *b*, *c*, *f*; 8 μ g, lane *d*) and total RNA (10 μ g, lanes *e*, *g*) were electrophoresed into a formaldehyde-1.2% agarose gel containing formaldehyde as a denaturant³⁶ and blotted onto a nitrocellulose filter³⁷. The following ³²P-labelled cDNA fragments were used as hybridization probes. Lane *a*, 258-bp *EcoRI/SmaI* (nucleotides 131-371) from α -subunit cDNA λ PIN- α 2; *b*, 157-bp *EcoRI/HindIII* (nucleotides 158-298) from β_A -subunit cDNA λ PIN- β_A 2; *c*, 231-bp *EcoRI/SmaI* (nucleotides 406-630) from β_B -subunit cDNA λ PIN- β_B 1; *d*, *e*, *EcoRI* insert of λ PIN- α 2; *f*, *g*, *EcoRI* insert of λ PIN- β_A 4. Filters were washed for 2 h with three changes of 0.1 \times SSC, 0.1% SDS at 60 °C.

cDNA cloning. From their respective band intensities, the α precursor mRNA is estimated to be in at least 10-fold higher abundance than the mRNA for the β_A precursor, and ~20-fold higher than the β_B precursor mRNA.

Using ribosomal RNAs as size standards, the α precursor mRNA, a single species, is found to be ~1,500 nucleotides long, in good agreement with the cloned cDNA sequence (Fig. 1*b*). β_A Precursor mRNA sequences are represented by two main species of ~4.5 and ~7.2 kb. The uniformly higher intensities of α and β_A species in polyadenylated than in total RNA (Fig. 3, lanes *d*-*g*) suggest that the β_A 4.5-kb species does not represent an artefact created by 28S RNA hybridizing to the cDNA probe. The β_A precursor cDNA sequences shown in Fig. 2*b* are therefore thought to be complementary to this 4.5-kb mRNA. The difference in size from the 3.6-kb-long cloned cDNA is attributed to missing 5' sequences. Unusually long 5'-untranslated regions are not without precedent²⁰⁻²². A differential splicing event or choice of a different polyadenylation signal might explain the existence of the 7.2-kb mRNA^{23,24}. The β_B precursor is encoded by a single mRNA of ~4.5 kb and is present at approximately half the level of the two β_A mRNAs.

The mature inhibin α - and β -subunits contain seven and nine cysteine residues respectively. Alignment of these residues shows that both types of subunits share a similar cysteine distribution and that some sequence homology exists around these residues (Fig. 4*a*), suggesting that α - and β -subunits are derived from one ancestral gene. Surprisingly, significant homology was found between the β -type sequence and the recently determined primary structure of human TGF- β ²⁰. As outlined in Fig. 4*b*, these peptides are of nearly equal length (inhibin β_A subunit, 116 residues; β_B subunit, 115; TGF- β , 112) and show a strikingly similar distribution of their nine cysteine residues. Using this cysteine 'pattern' for alignment, the β_A and TGF- β sequences



Fig. 4 Sequence homologies of inhibin subunits and TGF- β . *a*, Comparison of porcine inhibin β_A and α -subunit sequences. Numbers refer to the mature subunit sequences. *b*, Comparison of human TGF- β ²⁰ and porcine inhibin β -subunit sequences. Amino acids are shown in the one-letter code. Identical residues are boxed with cysteine residues shaded. Asterisks denote conservative changes.

have an additional 33 residues in identical positions and show conservative changes in more than 10 places. Similar homologies are seen on comparison of the β_B and TGF- β sequences. The β -subunit of inhibin is unlikely to be the porcine equivalent of human TGF- β , as there is almost absolute homology between human and murine TGF- β (R. Derynck, personal communication). Sequence homology extends into the pro-region where the inhibin β and TGF- β precursors²⁰ show similar hydrophobicity profiles (data not shown). Certain features of their cDNAs, in particular the highly G + C-rich sequence following the termination codons, may further attest to a common origin. Our findings strongly indicate that both inhibin subunits and TGF- β belong to one gene family and are biologically active as dimeric structures in which the subunits are linked by disulphide bridges^{14,25}.

The structural organization of porcine ovarian follicular fluid shares some intriguing features with the pituitary and placental glycoprotein hormones, FSH, luteinizing hormone (LH), thyroid stimulating hormone and chorionic gonadotropin²⁶. Like these molecules, inhibins A and B are high-*M_r* glycoproteins and have two subunits, one of which is common to both forms, the other variable, all encoded by different mRNA species. The mRNA encoding the common (α) subunit in both structures is in substantially higher abundance than the corresponding β -subunit mRNA, suggesting a similarity in the regulation of biosynthesis of each group of hormones²⁷. In the abovementioned hormones, the 'variable' (β) subunit confers the different biological specificities of each molecule, while the 'constant' (α) subunit presumably has a crucial role in folding and overall conformation of the complex²⁶. We have no evidence for differences in the biological properties of inhibins A and B. Unlike the known glycoprotein hormones, the two chains of both inhibins A and B are linked by disulphide bridges, a situation more closely resembling the immunoglobulins²⁸.

Regarding biosynthesis, each inhibin chain is released through proteolytic processing of a larger precursor, the α - and β -subunits representing the C-terminal region of each polypeptide. The discrepancy in size between the larger subunits of bovine¹⁶ and porcine inhibins can be readily explained by a different processing of their precursors.

The precursors for the porcine inhibin subunits have several common structural features: they carry the subunits at their C-termini, are similar in total size, have typical signal sequences at their N-termini and show a remarkably similar distribution of cysteine residues. In fact, closer analysis reveals significant sequence homology between the α - and β -subunits suggestive of an origin from distantly related genes. This, too, is a feature of the subunits constituting the pituitary and placental glycoprotein hormones²⁶.

Of considerable interest is the high homology of inhibin β -subunits with TGF- β , which points to an evolutionary link between the corresponding genes. It is intriguing that members of this distinctive gene family should be involved in such seemingly unrelated activities as the control of reproduction and cellular growth. What determines the activities of their respective protein products?

A clue may be found in the fact that TGF- β is active in a homodimeric form more homologous in amino-acid sequence to the β -subunits than to the α -subunit of inhibin. This fact and the analogy with the glycoprotein hormones noted above, direct attention to the β -subunits as the possible source of biological specificity of inhibin. Chain recombination experiments to generate novel heterodimers between inhibin and TGF- β subunits could test this hypothesis. Moreover, it is tempting to speculate that inhibin could function as a paracrine or autocrine growth regulator within gonadal tissue.

The prediction of the full inhibin structures from cloned cDNA sequences has allowed us to obtain specific antibodies against synthetic peptides based on these sequences, which are currently used to study inhibin regulation. The availability of cloned DNA sequences for inhibins will help to characterize the expression of these genes in gonads and possibly other tissues. More importantly, the recombinant expression of these sequences will allow the elucidation of their physiological role at the gonadal, pituitary and hypothalamic level.

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Epidermal growth factor receptor occupancy inhibits vaccinia virus infection

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Vaccinia virus encodes VGF, an early protein of relative molecular mass 19,000 (19K) which, from amino-acid residues 45 to 85, is homologous in 19 residues to epidermal growth factor (EGF), and transforming growth factor- α (TGF- α)^{1–3}. The conserved sequence includes a region of high homology (6 out of 10 amino acids) from residues 71 to 80, corresponding to the third disulphide loop of both EGF and TGF- α . This region has recently been shown to contain a binding region of TGF- α for the EGF receptor⁴, and this raises the question of whether vaccinia virus utilizes the EGF receptor in order to bind to and infect cells. We now show that occupancy of the EGF receptor inhibits vaccinia virus infection. Inhibition is observed in a dose-dependent fashion by pre-treatment with either EGF or synthetic decapeptide antagonists of EGF's mitogenic activity which correspond to the sequence of the third disulphide loop of VGF or TGF- α . The relative ability of the peptides to inhibit vaccinia virus infection parallels their binding affinity to the EGF receptor.

In order to test the concept that vaccinia virus makes use of the EGF receptor to infect cells, the effects of EGF and peptide antagonists of EGF were studied in a viral replication assay. Treatment of murine L-cells with EGF prior to the addition of vaccinia virus (strain WR; American Type Culture Collection) for a 1-h adsorption period inhibited viral infection in a dose-dependent manner, as monitored by a plaque reduction assay (Fig. 1). Further addition of EGF after the initial 1-h adsorption period and for the 4-day duration of the plaque assay resulted in a slight additional decrease in viral plaque number. Similarly, addition of synthetic decapeptide antagonists^{4,5} corresponding to the third disulphide loop of VGF or TGF- α (Fig. 2) resulted in a dose-related reduction in the number of viral plaques (Fig. 1). Further analysis of inhibition of single-cycle vaccinia virus replication by EGF of the synthetic decapeptides showed that whereas inhibition was achieved by EGF or decapeptides when the peptides were present before and during viral adsorption, no inhibition was observed when the peptides were added 2 h after viral adsorption. The additional decrease in viral plaque formation in the multi-cycle plaque assay when the peptides were also added 1 h after initial viral adsorption (Fig. 1) can be attributed both to inhibition of adsorption which was not complete in 1 h (which was confirmed in single-cycle virus yield experiments) and inhibition of viral adsorption in subsequent replication cycles.

The ability of EGF and the synthetic peptides to inhibit vaccinia virus replication correlated with their affinity for the EGF receptor on the L-cells (Fig. 3). EGF inhibited vaccinia virus plaque formation by approximately 50% at a concentration of 10^{-8} M (compared with 10^{-9} M for 50% maximal binding). The synthetic decapeptide antagonists (N and C terminal capped) which were approximately 10^3 times lower in EGF receptor

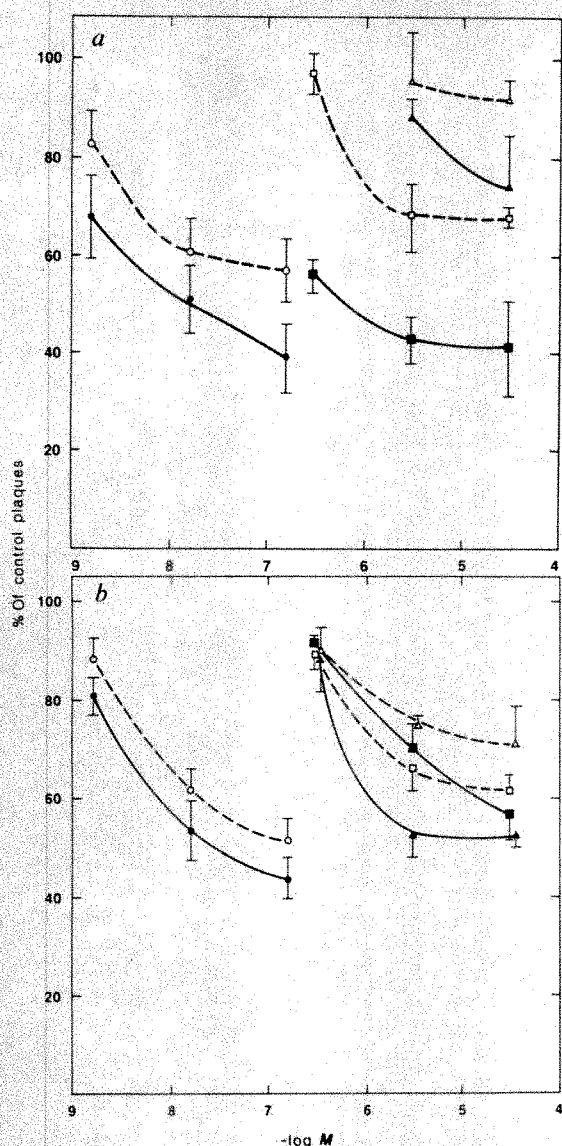


Fig. 1 Inhibition of vaccinia virus plaque formation by EGF and related synthetic decapeptides. Plaque reduction of vaccinia virus in L fibroblasts was performed as described elsewhere¹². EGF was purified from mouse submaxillary glands⁴. Peptide compounds were added to cells 3 h before viral adsorption as well as being left in the presence of cells during a 1-h viral adsorption period (open symbols); or were added both before and during viral adsorption as well as in the agar overlay after aspiration of the inoculum virus (solid symbols). Values plotted are the mean of three replicates \pm s.d. *a* = \circ , \bullet , EGF; \square , \blacksquare , acetyl TGF- α (34-43)-NH₂; \triangle , \blacktriangle , H-TGF- α (34-43)-OH (virus control was 38.8 ± 4.1 plaques, average of 6 replicates). *b* = \circ , \bullet , EGF; \square , \blacksquare , acetyl VGF(71-80)-OMe; \triangle , \blacktriangle , acetyl VGF(71-80)-NH₂ (virus control was 33.7 ± 1.0 plaques, average of 6 replicates).

affinity, showed a similar decreased potency relative to EGF for the inhibition of vaccinia virus replication. The corresponding 'free acid' decapeptide, which was less effective in binding to the EGF receptor, was similarly less effective in preventing vaccinia virus plaque formation. The fact that complete inhibition of vaccinia virus infections was not obtained at concentrations of EGF or peptides that were saturating in terms of binding to the EGF receptor suggests that, although initial interaction of VV with the EGF receptor may lead to productive infection, it is not the only pathway by which VV can infect L cells. Recently Stroobant *et al.*¹³ reported that VV was able to infect NR-G cells which lack detectable EGF receptors.

Specificity of the inhibition of vaccinia virus replication by EGF and the homologous synthetic peptides was confirmed by (1) lack of inhibition of vaccinia viral plaque formation or

		Amino-acid no.												
VGF	---	71	C	S	H	G	Y	T	G	I	R	C	--	
HuEGF	---	33	C	V	V	G	Y	I	G	E	R	C	--	
MuEGF	---	34	C	V	I	G	Y	S	G	D	R	C	--	
HuTGF- α	---	34	C	H	S	G	Y	V	G	A	R	C	--	
TGF- α (34-43) free acid			C	H	S	G	Y	V	G	A	R	C	-OH	
Acetyl TGF- α (34-43) ethylamide	Ac		C	H	S	G	Y	V	G	A	R	C	-NH ₂	
VGF(71-80) free acid			C	S	H	G	Y	T	G	I	R	C	-OH	
Acetyl VGF(71-80) amide	Ac		C	S	H	G	Y	T	G	I	R	C	-NH ₂	
Acetyl VGF(71-80) methyl ester	Ac		C	S	H	G	Y	T	G	I	R	C	-OMe	

Fig. 2 Peptide sequence of EGF, VGF and the TGF- α third disulphide loop, and sequences of the synthetic decapeptides. Sequence homologies between all peptides are boxed. TGF- α peptides and VGF peptides were synthesized using solid-phase techniques as described for the TGF- α peptides^{4,5}.

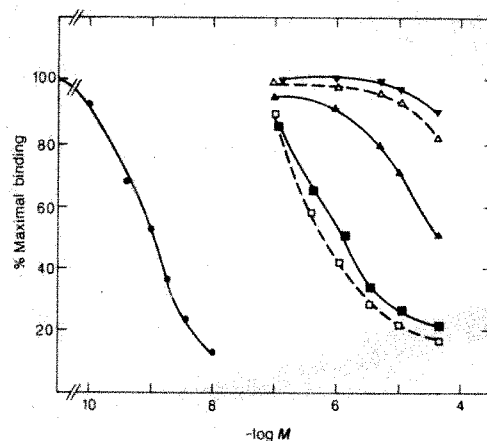


Fig. 3 Inhibition of binding of ¹²⁵I-EGF to L-cells. EGF was radioiodinated with Enzymobeads (Bio-Rad) to 120,000 c.p.m. per ng protein⁴. Receptor binding on L-cells was performed as previously described for A431 cells⁴. Nonspecific binding (determined in the presence of 100 times excess cold EGF) was $\leq 15\%$ of the specific binding; approximately 40,000-50,000 specific high-affinity binding sites were found per cell. L-cells were incubated at 4°C with 1 nM ¹²⁵I-EGF in the presence of EGF(\bullet), acetyl TGF- α (34-43)-NH₂(\square), H-TGF- α (34-43)-OH(\triangle), acetyl VGF(71-80)-OMe(\blacksquare), acetyl VGF(71-80)-NH₂(\blacktriangle) or H-VGF(71-80)-OH(\blacktriangledown). Average of 3 replicates plotted; s.d. $\leq 5\%$.

single-cycle virus yield by two unrelated polypeptides which bind to their respective receptors on L-cells, that is, insulin ($1 \mu\text{g ml}^{-1}$) or bovine growth hormone ($1 \mu\text{g ml}^{-1}$); and (2) no inhibition of two unrelated viruses by EGF or synthetic peptide (an RNA virus, vesicular stomatitis virus, Indiana strain, on L-cells; or a DNA virus, herpes simplex virus type II, strain G, on Vero cells).

It is noteworthy that EGF, which is an agonist in stimulating cellular DNA synthesis, and the synthetic decapeptides, which are antagonists of the mitogenic effect of EGF^{4,5}, both appear to inhibit vaccinia virus receptor interaction, with subsequent inhibition of viral replication. These results suggest that (1) it is likely that the inhibition occurs through blocking of the receptor site utilized by the virus and not by an indirect phenomenon after activation of the EGF-receptor system, and (2) a mitogenic effect of the native vaccinia virus 19K protein is not an obligatory requirement for viral replication. Recently, a vaccinia virus 25K protein (isolated from supernatants of

vaccinia virus-infected cells) was shown to bind to the EGF receptor and to have cellular mitogenic activity⁶. It remains to be demonstrated whether this protein corresponds to the 19K protein¹⁻³ bearing homology to TGF- α and EGF. It is possible that the growth factor secreted by vaccinia virus-infected cells may account for cellular hyperproliferation as previously suggested^{1,6}, as well as contribute to the self-limiting nature of vaccinia virus lesions.

The cellular receptors for certain viruses have been identified as being receptors for various other specific ligands. For example, Epstein-Barr virus infects B lymphocytes via the complement receptor CR2 (ref. 7); rabies virus may utilize the acetylcholine receptor⁸; the human T-cell lymphotropic virus type III (HTLV-III) receptor on T lymphocytes contains the CD4 (or T4) antigen^{9,10}; and the reovirus receptor has recently been identified as the cellular β -adrenergic receptor¹¹ (G. Gaulton, personal communication). Our results now provide another example of a virus that appears to utilize a cellular receptor specific for a physiological ligand.

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Characterization of receptors for human tumour necrosis factor and their regulation by γ -interferon

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Tumour necrosis factors, TNF- α and TNF- β (previously called lymphotoxin), are the products of activated monocytes and lymphocytes, respectively, and both have recently been purified, sequenced and cloned by recombinant DNA methods¹⁻⁵, revealing 35% identity and 50% homology in the amino-acid sequence. Both proteins have been found to be specifically toxic to many tumour cells. Furthermore, it has been reported that various interferons are synergistic with TNF for anti-tumour effects *in vitro*⁶⁻⁸, while activities attributed to the two proteins have also been shown to necrotize various tumours *in vivo*^{2,3,9}. We have now prepared ¹²⁵I-labelled highly purified recombinant human TNF- α to study in detail its binding to the human cervical carcinoma cell line ME-180. Our results indicate that there is a single class of specific high-affinity receptors for TNF on this cell line which has a K_d of about 0.2 nM and an average of 2,000 receptor sites per cell. The binding of labelled TNF- α to these cells can be inhibited by both TNF- α and TNF- β but not by γ -interferon (IFN- γ). However, preincubation of cells with IFN- γ increases the total number of TNF receptors two to threefold without any significant change in the affinity constant. This is the first report that TNF- α

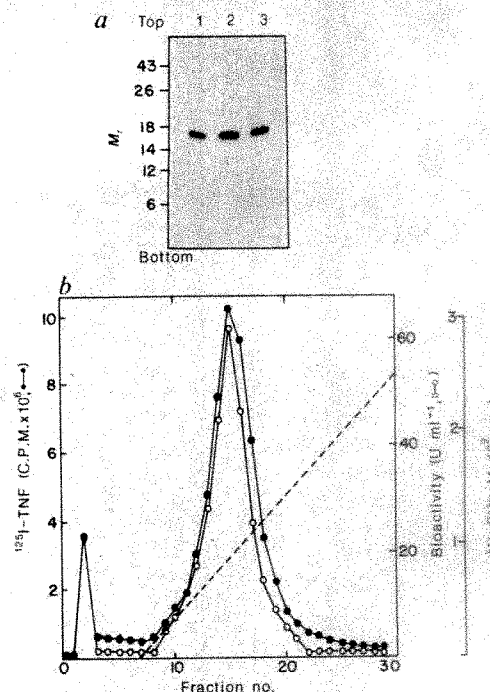


Fig. 1 SDS-polyacrylamide gel electrophoresis (a) and Mono Q-fast protein liquid chromatography of iodinated TNF- α (b). The autoradiographs shown in a represent samples from three different iodinations (lanes 1-3). About 10,000 c.p.m. of ¹²⁵I-TNF were analysed. The M_r s ($\times 10^3$) of protein standards run in parallel are indicated.

Methods. Purified TNF (10 μ g) was radioiodinated by the iodogen method¹⁰. 0.1 M phosphate buffer (pH 7.4) was added to a glass tube coated with 5 μ g of iodogen (Pierce) and incubated with 1 mCi of Na ¹²⁵I for 10 min at 4 °C. Then the mixture was transferred to a tube containing TNF- α and allowed to react for 5 min at 4 °C. The reaction was stopped by removal of the soluble material which was separated from free iodide by filtration with Sephadex G-25 that had been equilibrated with 0.1 M phosphate buffer containing 0.1% gelatin. Sephadex G-25-purified material was characterized further on a Mono Q-column equilibrated in 25 mM Tris pH 7.4 containing 0.05% Tween 20 (b). Linear salt gradients of 0-0.25 M NaCl in equilibration buffer were used for elution of ¹²⁵I-TNF. The column was eluted at a flow rate of 1 ml min⁻¹ and 2 ml per fraction was collected. All these operations were carried out at room temperature. The column fractions were stored at 4 °C and analysed for radioactivity and cytolytic activity. The latter was measured by the lysis of actinomycin D-treated mouse fibroblast L929 cells (30,000 cells per microtitre well) plated in monolayer and quantitated by dye uptake⁷. The protein concentration of iodinated TNF was determined by immunoassay for TNF- α . Column fractions were also analysed by electrophoresis on 15% polyacrylamide slab gels¹⁷.

and - β share a common receptor and that the receptors can be up-regulated by interferon. Our results may explain previous observations regarding similar biological activities observed for these two cytotoxic proteins and also their synergistic action with interferons.

TNF- α and - β have been cloned and expressed by recombinant DNA methods in *Escherichia coli*. The proteins were purified to homogeneity from bacteria either by immunoaffinity purification using monoclonal antibodies or by ion-exchange chromatography as described previously¹⁻⁵. Both proteins were homogeneous by the criteria of amino-terminal sequence analysis and SDS-polyacrylamide gel electrophoresis. TNF- α and - β have relative molecular masses (M_r s) of 17,000 and 15,500, respectively, and specific activities of $\sim 100 \times 10^6$ units per mg as determined by lysis of actinomycin D-treated mouse L929 cells⁷.

The labelling of TNF- α and - β by chloramine-T, lactoperoxidase and Bolton-Hunter reagent methods provided ¹²⁵I-TNF with low specific activity and resulted in some cases in a >90% decline in biological activity. However, when TNF- α

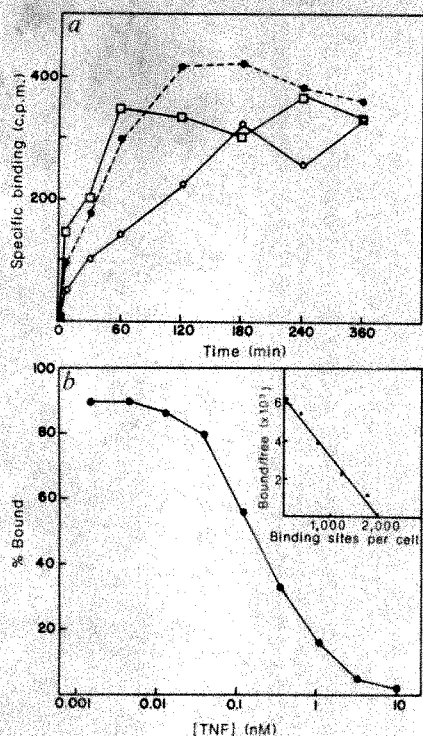


Fig. 2 *a*, Time course of ^{125}I -TNF binding at 4 °C (○), 23 °C (●) and 37 °C (□) and *b*, competition curve of labelled bound TNF- α with unlabelled TNF- α . For each time point in *a*, 0.2×10^6 ME-180 cell monolayers were incubated in a final volume of 0.5 ml of McCoy's 5A medium containing 10% fetal bovine serum at the indicated temperature with 0.2×10^6 c.p.m. of ^{125}I -TNF- α in the presence and absence of 100 nM unlabelled TNF- α . Each data point represents the mean of triplicate determinations. After incubation, cell monolayers were washed three times with the incubation buffer, solubilized with 2% SDS and cell-bound radioactivity was determined in a γ -counter. In *b*, cells were incubated at 37 °C for 2 h with 0.2×10^6 c.p.m. of ^{125}I -TNF- α and indicated amounts of unlabelled TNF- α . Nonspecific binding was $\leq 10\%$ of total binding. The data are plotted as specific binding against concentration of unlabelled TNF- α . Inset, a Scatchard plot of the data.

was labelled by the iodogen method¹⁰, a fully biologically active ^{125}I -TNF- α was obtained which was stable for more than 4 weeks. ^{125}I -TNF- α had a specific activity of ~ 804 Ci per mmol protein, and $\sim 50\%$ incorporation of the label into the protein was observed. Approximately 50% of the TNF- α molecules were labelled, assuming monoiodination by this method. TNF- α contains seven tyrosine residues. Digestion of ^{125}I -TNF- α by trypsin (5% w/w) provided a single radioactive peptide. The amino-acid sequence of this peptide indicated that the tyrosine residue at position 87 was radiolabelled.

The product of this iodination reaction showed a single- M_r component of $\sim 17,000$ (TNF- α has a M_r of 17,100 as calculated from the protein sequence) when analysed by gel electrophoresis (Fig. 1a). Fractionation of ^{125}I -TNF- α by gel filtration chromatography on a TSK column also provided a single peak of radioactive protein which co-eluted with the biological activity (data not shown). The apparent M_r of labelled TNF- α by gel filtration was found to be $\sim 20,000$, similar to that determined for unlabelled recombinant TNF. A single peak of radioactive protein also co-eluted with biological activity when chromatographed by Mono Q-fast protein liquid chromatography (Fig. 1b), showing a retention time similar to that of unlabelled TNF.

We investigated the interaction of ^{125}I -TNF- α with cellular binding sites by incubating it with monolayers of human tumour cells in tissue culture. After incubation, the cells were washed three times with incubation medium, solubilized with 2% SDS and the radioactivity bound to the cell monolayer was measured. To avoid a species-specific barrier, human tumour cells were used to determine the binding of human TNF- α . It has been

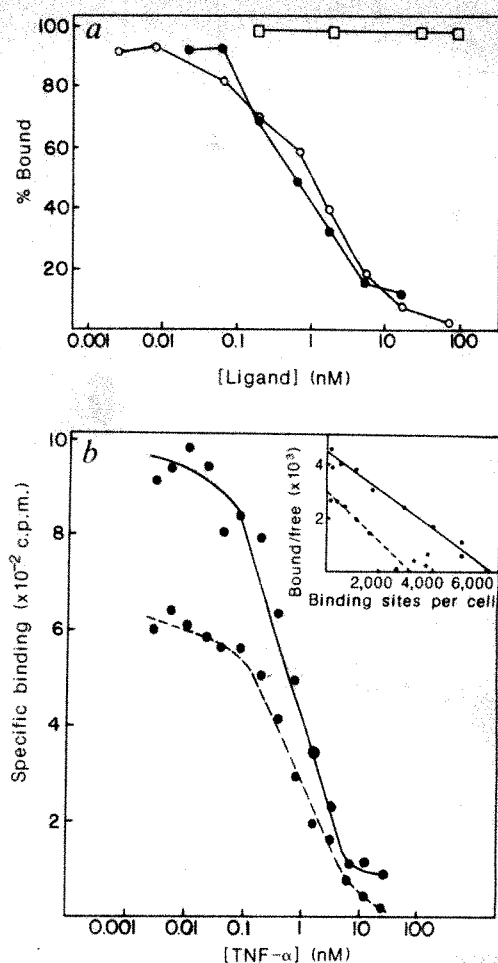


Fig. 3 *a*, Competition of TNF- α , TNF- β and IFN- γ with ^{125}I -TNF- α for binding to ME-180 cells; *b*, competition of TNF- α with ^{125}I -TNF- α for binding to ME-180 cells before and after exposure to IFN- γ . Incubation conditions for *a* were as described for Fig. 2a, with the addition of the indicated concentrations of the following: TNF- α (●), TNF- β (○) and IFN- γ (□). The results are plotted as specific binding relative to an assay with no added competitor. In *b*, ME-180 monolayer cells were preincubated overnight in the absence (---) and presence (—) of $1 \mu\text{g ml}^{-1}$ of IFN- γ , then washed twice and examined for TNF- α binding as described for Fig. 2a. Inset, a Scatchard plot of the data.

reported previously that a human cervical carcinoma cell line, ME-180, is a very sensitive target for TNF², and we therefore used this cell line to study TNF binding in all experiments. All binding assays were carried out using a 0.5-ml total incubation volume layered on 0.2×10^6 cells plated as a monolayer. Binding was quite reproducible, each preparation of labelled TNF- α giving essentially the same results when tested in standard conditions over several weeks.

Figure 2a shows the time course of binding at 4, 23 and 37 °C. An initial increase in the rate of ^{125}I -TNF- α binding was observed with increase in temperature, reaching a plateau after 1 h, 2 h and 3 h at 37, 23 and 4 °C, respectively. The total binding was almost the same at all three temperatures. All subsequent experiments were carried out at 37 °C, the temperature of the cell cultures. The binding of ^{125}I -TNF- α to the cells was inhibited by various concentrations of unlabelled TNF- α ; Scatchard analysis of these data gave an apparent dissociation constant of 0.2 nM (Fig. 2b). The average number of binding sites per cell was $\sim 2,000$. These observations are consistent with the binding of ^{125}I -TNF- α to cellular receptors, although the identification of a specific receptor would require its characterization as a macromolecular complex with TNF and its purification.

Specificity of binding was examined by competition with highly purified recombinant preparations of TNF- β and IFN- γ . The competition with unlabelled TNF- β could be superimposed

on that obtained with TNF- α , whereas IFN- γ did not compete even at 1,000-fold excess concentration (Fig. 3a). It seems, therefore, that both TNF- α and - β compete with 125 I-TNF- α for binding to the same receptors.

It has been reported that IFN- γ is synergistic with TNF for anti-tumour action on many cell lines⁶⁻⁸. To understand the mechanism of this synergism, we pretreated the cells overnight with $1 \mu\text{g ml}^{-1}$ of IFN- γ and compared 125 I-TNF- α binding with untreated cells in a competition assay. Figure 3b shows the competition curve with IFN- γ -treated cells in relation to untreated cells in similar conditions. Scatchard analysis of the two curves indicated no significant change in the affinity constants, but a two to threefold increase in the number of receptors per cell was noted with interferon-treated cells. The increase in receptor number was observed consistently in four sets of experiments. Pretreatment of cells with $1 \mu\text{g ml}^{-1}$ of IFN- α was less effective than IFN- γ in inducing TNF receptors (data not shown).

Our experiments measured TNF binding at 37 °C. At this temperature, TNF may interact with cellular receptors in a complex way, possibly as a result of a series of metabolic events. This is suggested by our observation that the binding of 125 I-TNF after 2 h at 37 °C could not be reversed by either trypsin or salt and pH elution. However, a partial reversal (<30%) in binding was observed after 15 min (data not shown). At 4 °C, two-thirds of the total binding of 125 I-TNF could be reversed after 15 min. A similar kind of irreversible binding to that described here has been reported previously for receptors for platelet-derived growth factor¹¹. Furthermore, various characteristics of TNF binding reported here are very similar to those described for other polypeptide hormones including interferons¹¹⁻¹³. This includes a single class of specific high-affinity binding sites possessing a limited number of receptors per cell.

The inhibition of TNF- α binding by TNF- β may explain several common biological effects *in vitro* and *in vivo* observed for the two molecules⁸⁻¹⁴. Our experiments suggest that the receptor binding domains of TNF- α and - β should be similar. Two particularly conserved regions occur at amino acids 11-20 and 121-126 (TNF- α numbering) where all residues are homologous to TNF- β . It is possible that these regions of both proteins are involved in interaction with receptors. IFN- γ is known to exhibit synergistic biological effects with several other lymphokines including IFN- α , IFN- β , interleukin-2, TNF- α and - β (refs 7, 8, 15, 16). The mechanism of such synergistic action is unknown. This is the first report of an IFN- γ -induced increase in the total number of TNF receptors. It also suggests that the receptors are central to the action of TNF. Studies are now in progress to characterize TNF receptors further and to elucidate various biochemical events which follow receptor binding and precede cell death.

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Selective immortalization of murine macrophages from fresh bone marrow by a *raf/myc* recombinant murine retrovirus

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Myeloid precursors can be grown *in vitro* in the presence of specific growth factors; however, their expansion is limited by a competing process of terminal differentiation^{1,2}. Proto-oncogenes seem to be involved in cellular proliferation and/or differentiation and may also play a role in the myelopoietic process^{3,4}. Murine myeloid precursors which are grown *in vitro* with growth factors respond with augmented self-renewal upon infection with recombinant retroviruses carrying the *v-myc* or *v-src* oncogenes⁵⁻¹¹, suggesting a synergism or complementation between some viral oncogenes (*v-onc*) and certain growth factors. We now show that the combination of two *v-onc* genes (*raf* and *myc*) induces the selective proliferation of monocytic cells from fresh murine bone marrow (BM) in the absence of a specific growth factor supplement. Depending on the culture conditions these cells can either differentiate and cease to proliferate or grow continuously, thus mimicking the alternative pathways that can be followed by committed BM stem cells *in vivo*.

Mononuclear BM cells obtained from femurs of C3H/HeJ mice were cultured in standard medium after overnight infection with retroviruses carrying *v-raf* and/or *v-myc* oncogenes. Untreated BM cells as well as cells infected with helper virus (Fig. 1a) or with retroviruses carrying only *v-raf* (3611, J1) or *v-myc* (J3, J5) (data not shown) died after a few days of culture. In contrast, in the cultures infected with the virus carrying both *v-raf* and *v-myc* (J2), clusters of non-adherent proliferating cells (J2-BM cells) could be detected 7-10 days after infection. J2-BM cells reached a peak in cell number and rate of proliferation 14 days after infection (Fig. 1a, b). At the same time, we observed a peak in frequency of colony-forming cells in soft agar (Fig. 1c). The decrease in cellular proliferation was not caused by lack of viral replication in J2-BM cells, since the fraction of virus-positive cells, as determined by infectious centre assay¹², was constant for at least 35 days following infection (Fig. 1d).

J2-BM cells growing in suspensions 14 days after infection (day 14 J2-BM cells) were characterized. Morphological examination of Giesma-stained cells revealed a relatively homogeneous cell population (Fig. 2a). The clonal expansion of day 14 J2-BM cells was further indicated by Southern blot analysis of J2-virus integration sites. The presence of a single virus-cell junction fragment (data not shown) in the DNA from the infected cultures strongly suggests that J2-BM cells are of clonal origin.

The monocytic nature of J2-BM cells was established by cytochemical and functional markers. J2-BM cells expressed nonspecific esterase, were phagocytic (Fig. 2b), and produced lysozyme ($\geq 8 \mu\text{g per } 10^5$ cells per 72 h). No peroxidase activity was detected. Flow cytometry analysis confirmed that J2-BM cells belong to the monocytic lineage (Table 1), since more than 95% were MAC-1-positive and 85% expressed Fc receptors. A minimum of 50% of the cells expressed the mouse heat-stable antigen, asialo-GM1, glycolipid and F4/80 antigen. In contrast, day 14 J2-BM cells were consistently negative for T-cell (Ly 1.1, Lyt 2.1, L3T4) and B-cell (surface immunoglobulins) markers.

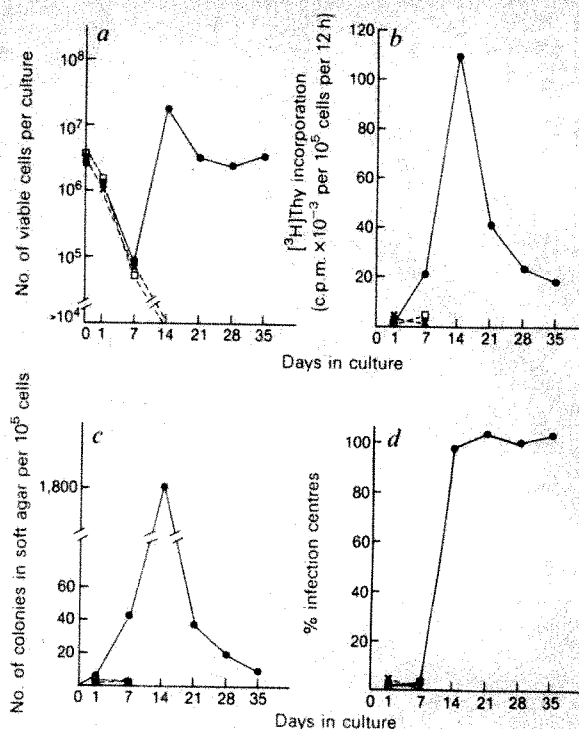
Day 14 J2-BM cells expressed helper and transforming virus-specific functions, being highly positive for production of trans-

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Fig. 1 Kinetics of cell growth, ^3H -thymidine (^3H -Thy) incorporation, formation of colonies in soft agar, and % infectious centres in BM cells after infection. A leukaemogenic strain of Moloney murine leukaemia virus was used as helper virus. The results are from representative experiments. Cell growth (a), ^3H -Thy incorporation (b), formation of colonies in soft agar (c), and % infectious centres (d) were monitored weekly in uninfected controls (\square), and in BM cells infected with J2 virus plus helper virus (\bullet) or with helper virus alone (\times). Each point in b and c represents the mean of triplicate samples. The standard errors were less than 5% of the mean value.

Methods. The BM cells used in these studies were obtained from femurs of C3H/HeJ mice. This strain was used as a source of BM cells because their macrophages do not respond to endotoxins which often contaminate tissue culture media and stimulate endogenous production of growth factors in BM cultures¹⁹. 5×10^6 fresh BM cells, obtained by density separation on Ficoll²⁹, were resuspended in standard medium [Dulbecco's modified Eagle's medium supplemented with penicillin (100 units ml^{-1}), streptomycin (100 $\mu\text{g ml}^{-1}$), glutamine (4 mM) and 10% fetal bovine serum] and incubated overnight in the presence of control supernatants or of viruses containing supernatants. The viruses used were the following: (1) 3611 Moloney sarcoma virus³⁰ (containing *v-raf*); (2) J1 containing a complete *raf/mil* hybrid oncogene and the 5' half of MH2 *v-myc*^{31,32}; (3) J2 (which contains a complete *raf/mil* hybrid oncogene and a complete *myc* gene consisting of the 5' half of the MH2 *v-myc* and the 3' half of MC29 *v-myc*)^{30,31}; (4) J3 (derived from J2 by a 200-base pair deletion which takes the *raf/mil* gene out of the reading frame, *v-myc* remaining functional)³²; (5) J5 (which expresses a *v-myc* gene derived entirely from MC29)³².

The infections were performed at a viral concentration ranging from 1 to 5×10^4 particles per ml, as determined by fluorescence focus induction assay with anti-*v-myc*-antibodies³¹ in the case of *v-myc* carrying retroviruses J3 and J5, or by focus-forming assays in the case of *v-raf*-carrying viruses 3611 and J1. ^3H -Thy incorporation was determined in 10^5 cells pulsed with $10 \mu\text{Ci ml}^{-1}$ of [^3H]Thy (NEN) for 12 h at 37 °C. The cultures in soft agar were performed as described previously¹⁹ and the colonies (>50 cells) were scored after 10 days in culture. The infectious centres of mitomycin-treated BM cells were determined on 3T3 fibroblast indicator cells¹².



forming virus (Fig. 1d), for envelope glycoprotein gp70 (Table 1) and for *v-myc*- and *v-raf*-derived proteins. More than 90% of J2-BM cells, in fact, showed a specific nuclear staining (Fig. 2c) with anti-*v-myc* antibodies¹³ and a specific cytoplasmic fluorescence (Fig. 2d) with anti-*v-raf* antibodies¹⁴. Because of the cross-reactivity of the anti-*v-myc* or anti-*v-raf* antibodies with cellular *myc* or *raf* proteins, J2-BM cells were tested for expression of viral and cellular messenger RNA by Northern blot analysis and compared with normal or J2-virus-transformed NIH 3T3 fibroblasts. An MC29 *v-myc* DNA probe detected high levels of *v-myc* mRNA in both J2-BM cells and transformed

fibroblasts (Fig. 3a). Similarly, high expression of *v-raf* was detected using a cross-reacting human *c-raf* probe (Fig. 3b). When the blot was hybridized with a murine *c-myc* probe, which under these conditions does not recognize *v-myc*, no expression of *c-myc* was detected in J2-virus-transformed fibroblasts or J2-BM cells (Fig. 3c). These results show that J2-BM cells actively synthesized large amounts of viral *myc* and *raf* mRNA and that *c-myc* mRNA was undetectable. Therefore, the *myc* protein detected by immunofluorescence is probably of viral origin.

Infections of BM cells with J2 virus have consistently resulted

Table 1 Cell-surface markers of J2-BM cells (day 14)

Surface marker*	% Positive J2-BM cells†	Distribution
MAC-1 (M1/70)	96.8 (451)	Macrophages, granulocytes ²⁰
Fc receptor (2.4G2)	85.7‡ (284)	Macrophages, B cells ²¹
Heat-stable antigen on mouse blood cells (M1/69)	64.5§ (284)	B cells, macrophages, red blood cells, thymocytes, BM cells ²²
Asialo-GM ₁ glycolipid (anti-asGM ₁)	45.0§ (152)	Macrophages, natural killer cells, fetal thymocytes ²³
F4/80 (F4/80)	44.6§ (149)	Mononuclear phagocytes ²⁴
Ly 1.1 (39/3)	2.8 (101)	Pan-T cells, some B cells ²⁵
Lyt 2.1 (clone 49-31.1)	0.4 (93)	T-cell subsets, thymocytes ²⁶
L3T4 (H129. 19)	3.2 (105)	T-cell subsets, thymocytes ²⁷
Surface immunoglobulin (fluoresceinated goat anti-mouse [GAM]Ig)	0.7 (105)	B cells
Surface glycoprotein gp70 (37594)	94.6 (429)	Viral protein on mammalian cells ²⁸

Cells (10^6 in 50 μl) were incubated for 40 min at 4 °C with 10–50 μl of appropriately diluted antibodies or with control supernatants. They were then washed three times and incubated for 40 min at 4 °C with 10 μl of appropriately diluted fluoresceinated anti-immunoglobulins (Cappel Laboratories). After another three washes the cells were analysed for immunofluorescence on a Cytofluorograf System 30-H with a 2150 computer (Ortho Diagnostic) capable of simultaneous measurement of forward and right-angle light scatter (488 nm), red (>600 nm) and green (530 nm) fluorescence. Cells were illuminated by a 4-W argon laser (Lexel) emitting 500 MW of 488-nm light. Cells analysed for immunofluorescence were selected by forward and right-angle light scatter. Dead cells less than 1% were identified by the uptake of propidium iodide (red fluorescence) and excluded from green fluorescence analysis. A green fluorescence histogram of 1,000-channel resolution was collected from 10,000 viable cells for each sample analysed. Both unstained cells and cells treated with fluorescent second antibodies were used as negative controls for these experiments.

* The designations of the antibodies used are indicated in parentheses.

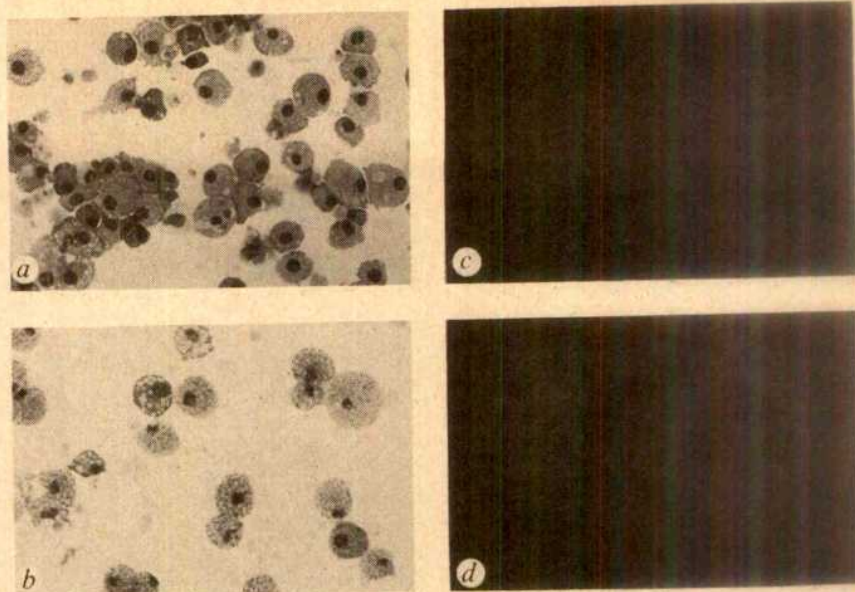
† In parentheses are shown the relative mean fluorescence units measured on ~1.5 decade log scale with 1,000 channels of fluorescence.

‡ The high percentage of Fc receptor-bearing cells was also confirmed by enumeration of rosette-forming cells²¹ with IgG-coated red cells (data not shown).

§ These percentages represent the mean number of positive cells from three experiments. Although the net calculation of positive cells is ~50%, greater than 90% of the stained cells expressed a fluorescence intensity above the mean fluorescence of the negative controls.

Fig. 2 Photomicrographs of J2-BM cells. *a*, Morphology of Giesma-stained J2-BM cells; *b*, phagocytosis of opsonized zymosan by J2-BM cells 14 days after infection with J2 virus. *c*, *d*, Specific staining of *myc*- and *raf*-derived proteins in J2-BM cells 14 days after infection. Note nuclear fluorescence (*c*) and cytoplasmic fluorescence (*d*) in cells stained with anti-*myc* serum¹³ or anti-*raf* serum¹⁴, respectively.

Methods. For *a* and *b* 10^6 cells from a 2-week-old culture were incubated for 16 h in standard medium or in the presence of zymosan ($50 \mu\text{g ml}^{-1}$) opsonized with normal mouse serum. The cells were then washed and cytospin preparations were stained with Giesma. Cells which had ingested more than three zymosan particles were considered positive. For *c* and *d*, cytospin preparations of J2-BM cells were fixed in acetone for 10 min, stained for 40 min with anti-*myc* (provided by Dr Karin Moelling) or anti-*raf* sera, washed three times in phosphate-buffered saline (PBS), stained for an additional 40 min with appropriately diluted fluorescein-conjugated anti-rabbit serum (Cappel) and washed three times. Less than 1% of fresh BM cells showed very weak reactivity with either antibody, whereas J2-transformed fibroblasts displayed a pattern and intensity of fluorescence similar to that of J2-BM cells (data not shown). Slides were examined and photographed at 400- and 1,000-fold magnifications using a Zeiss microscope (made available by Dr U. Saffioti).



in selective growth of J2-BM cells at day 14. Following the initial proliferative phase associated with the presence of free floating clusters of cells (Fig. 4*a*), J2-BM cells formed an adherent monolayer (Fig. 4*b*) and ceased to proliferate. Adherent J2-BM cells were still virus producers, expressed *raf*- and *myc*-derived proteins and were morphologically indistinguishable from normal BM-derived macrophages (data not shown). Therefore, it appears that the proliferative stimulus of the J2 virus, which caused the *in vitro* expansion of the monocytic cells, is limited by a dominant differentiation process.

These considerations raised the question as to whether the differentiation was terminal, as in normal BM-derived macrophages, or whether J2-BM cells could revert to proliferating cells. We found that differentiated J2-BM cells could be induced to proliferate again by addition of dextran-based beads (CT; Cytodex, Pharmacia) to the 35-day cultures. Following addition of the beads, in four out of five experiments performed, the cells detached from plastic, became round and loosely adherent to the beads and formed floating cultures (Fig. 4*c*), which have been kept in continuous proliferation for more than 8 months.

J2-BM cells cultured with beads (J2-BM-CT cells) were clonable in soft agar (with an efficiency of 30–50%), indicating that their *in vitro* growth was an intrinsic property of individual cells rather than a proliferation mediated by the trophic activity of feeder cells. The plating efficiency of the J2-BM-CT cells, higher than that of the day 14 J2-BM cells (1.8%), could be the result of a progression step in the establishment of permanent cell lines from the initially infected cultures. Eighteen double-selected clones were tested for surface markers and found to be identical to each other (data not shown) and indistinguishable from the day 14 J2-BM cells (Table 1). Furthermore, all five clones tested so far produced transforming virus, suggesting that both oncogenes were active in the cloned cell lines.

Both *v-myc* and *v-raf* genes had to be expressed to induce proliferation of BM cells in standard medium, since viruses carrying either oncogene alone did not promote BM cell growth. The expression of both *v-myc* and *v-raf* therefore might have overridden the need for specific exogenous growth factor. This is consistent with earlier observations in the avian system where it was found that viruses carrying only *v-myc* augment the response of primary BM macrophages to growth factors^{9,10,16}. Moreover, in the murine system, infection of interleukin-3 (IL-3)-responsive BM cells with virus carrying *v-raf* induced their continuous *in vitro* proliferation¹⁷, whereas infection with virus carrying *v-myc* caused abrogation of IL-3 requirement¹⁸. It is possible that *v-myc* substitutes for growth factors in the prolifer-

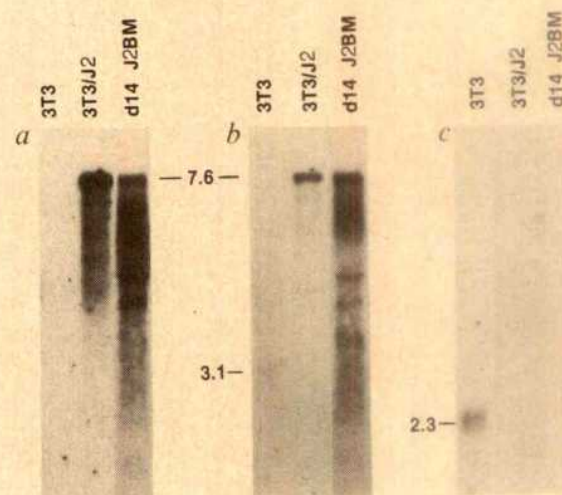


Fig. 3 Northern hybridization analysis of J2-BM cells 14 days after infection (d14 J2BM) and of J2-transformed NIH 3T3 fibroblasts (3T3/J2). Polysome-associated RNA was isolated³³ and poly(A)⁺-containing RNA selected using oligo(dT)-cellulose chromatography. This RNA (15 μg) was denatured by glyoxalation³⁴, separated electrophoretically in agarose gels, transferred to nitrocellulose and hybridized under stringent conditions to ³²P-labelled nick-translated DNA probes¹⁵. *a*, *v-myc* hybridizations. The probe was a 1.4-kilobase (kb) *Pst*I/*Aha*III *v-myc*-specific fragment of the MC29 provirus^{35,36}. The intensity of hybridization with these RNAs varied considerably; the 3T3 and 3T3/J2 samples were exposed for 1 week whereas the d14 J2BM was exposed for 20 h. *b*, *raf* hybridizations. The probe was a 2.97-kb human *c-raf*-1 cDNA³⁷. The autoradiograph was exposed for 4 days. *c*, *c-myc* hybridizations. The probe was a 5.6-kb *c-myc* *Bam*HI fragment from subclone pS107 (ref. 38). The autoradiograph was exposed for 1 week. Sizes of RNAs were determined from their migration relative to that of ³²P-labelled denatured *Hind*III fragments of bacteriophage λ . The 7.6-kb RNA is the genome-size RNA of the J2 virus³²; several smaller subgenomic RNAs are also transcribed from this provirus. The 3.1-kb transcript is the endogenous *c-raf*-1 mRNA³⁹ whereas the 2.3-kb RNA is the *c-myc* transcript⁴⁰.

ation of J2-cells. In fact, we did not detect any colony-stimulating activity in virus-free supernatants from J2-BM-CT cells, as determined by standard colony-forming assay¹⁹ with either fresh mouse BM or day 14 J2-BM cells (data not shown).

Only J2-BM cells, but not normal macrophages, could be rescued from the resting differentiated stage and reverted to

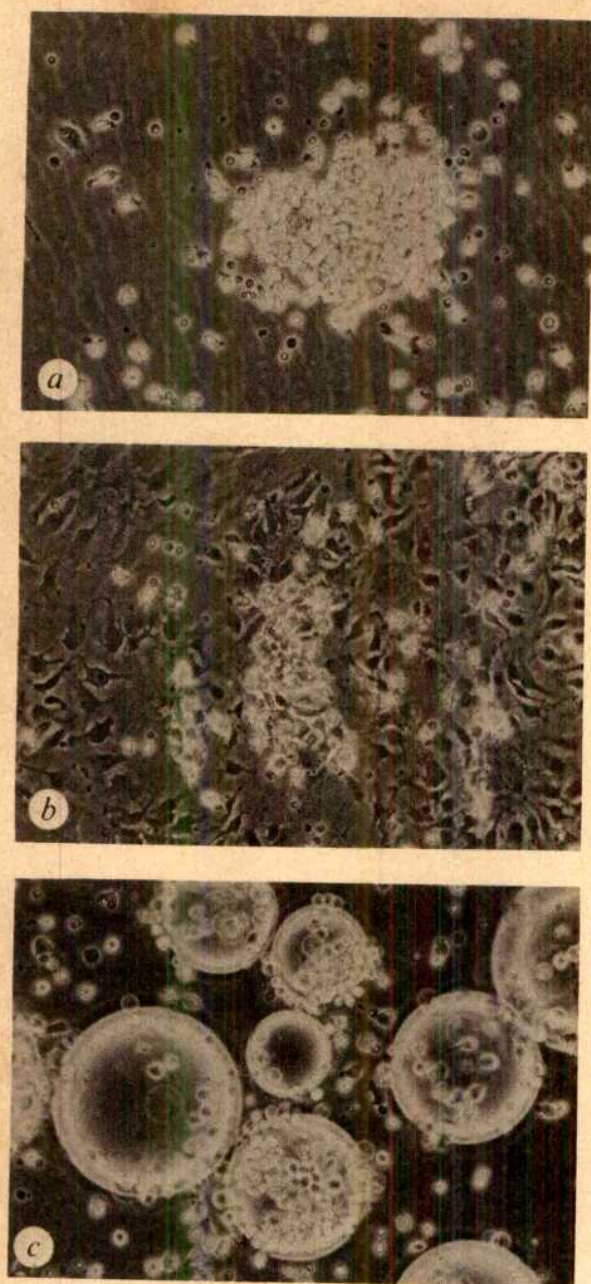


Fig. 4 Photomicrographs of J2-BM cells maintained in liquid cultures. *a*, Morphology of day 14 J2-BM cells. *b*, Morphology of J2-BM cells 35 days after infection. *c*, Morphology of J2-BM cells maintained in active proliferation by culturing them with dextran-based beads. The cells were photographed *in situ* after gently removing most of the supernatants, at 160-fold magnifications using a Zeiss microscope.

proliferating cells by co-culture with beads, suggesting that the response to the growth-promoting activity of *v-raf* and *v-myc*, down-regulated by differentiation, could be reactivated. In our system, three signals seem to be required for immortalization, defined as continuous *in vitro* growth of J2-BM cells: the two oncogenes *v-myc/v-raf* and the beads. The mechanism underlying reversal of differentiation by addition of dextran-based beads is unclear. One possibility that we are testing is the absorption of differentiating factors onto their charged surface. Identification of factors involved in the induction of differentiation could provide insights into the cellular resistance to the transforming activity of oncogenes.

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Domain structure of human glucocorticoid receptor and its relationship to the *v-erb-A* oncogene product

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The interaction of steroids with their nuclear receptors induces a cascade of regulatory events that results from the activation of specific sets of genes by the hormone/receptor complex¹. Steroids, either acting alone or possibly synergistically with other growth factors, can influence the DNA synthesis and proliferation of specific target cells, initiate developmental pathways and activate expression of the differentiated phenotype²⁻⁷. Moreover, steroid hormones have been implicated in abnormal growth regulation both in tumours and tumour-derived cell lines^{8,9}. The identification of complementary DNAs encoding the human glucocorticoid receptor (hGR) predicts two protein forms (α and β ; 777 and 742 amino acids long, respectively) which differ at their carboxy termini¹⁰. We report here that both forms of the receptor are related, with respect to their domain structure, to the *v-erb-A* oncogene product of avian erythroblastosis virus (AEV), which suggests that steroid receptor genes and the *c-erb-A* proto-oncogene are derived

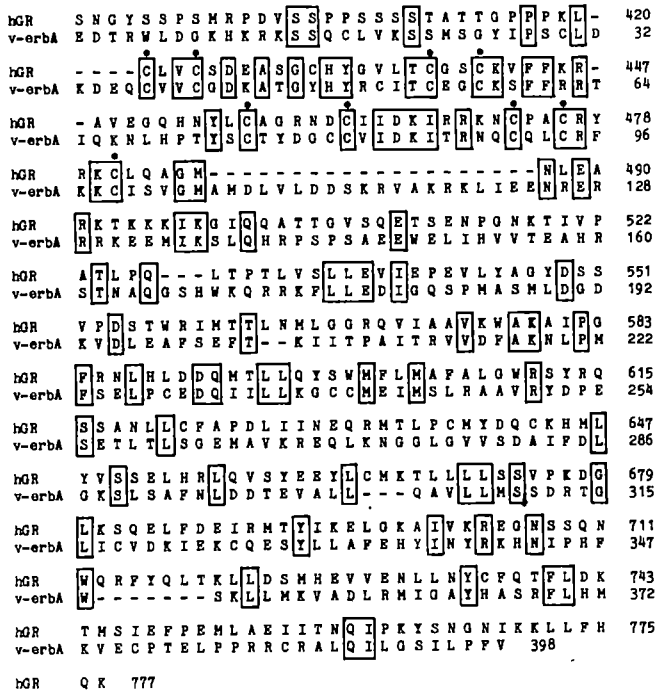


Fig. 1 Amino-acid sequence alignment of the carboxy-terminal half of the human glucocorticoid receptor (hGR) and *v-erb-A* oncogene product. Amino-acid residues 370-777 from hGR were aligned with amino-acid residues 1-398 from p75^{gag-erb-A}; identical residues are boxed and gaps are indicated by dashes. Matching cysteine residues are indicated by dots above the sequence.

from a common primordial regulatory gene. Therefore, oncogenicity by AEV may result, in part, from the inappropriate activity of a truncated steroid receptor or a related regulatory molecule encoded by *v-erb-A*. This suggests a mechanism by which *trans*-acting factors may facilitate transformation. We also identify a short region of hGR that is homologous with the *Drosophila* homoeotic proteins encoded by *Antennapedia* and *fushi tarazu*.

In the accompanying paper, we have deduced the entire amino-acid sequence of the human glucocorticoid receptor by nucleotide sequence analysis of overlapping cDNA clones¹⁰. The amino-acid sequence was scanned against a protein database maintained by R. Doolittle (University of California, San Diego) and a remarkable homology was found between the carboxy-terminal half of the receptor and the putative oncogene protein p75^{gag-erb-A} from AEV¹¹. Comparison of the last 387 amino acids of the receptor with the terminal 395 amino acids of the *gag-erb-A* fusion polypeptide revealed 85 matches, with 22% amino-acid identity overall (Fig. 1). The Needleman-Wunsch similarity value for the comparison was greater than 10 standard deviation units above the mean of 30 shuffled sequences, signifying statistically significant relatedness^{12,13}. In addition, 67 conservative amino-acid substitutions were found throughout this comparison interval. There is even greater identity (40%) between amino-acid residues 421 and 481, which corresponds to a cysteine/lysine/arginine-rich portion of the receptor. Nine out of ten cysteine residues of hGR are conserved in this region of maximal homology with *erb-A*. This is particularly significant because cysteines are not normally abundant and may participate in polypeptide tertiary structure.

In the amino-terminal half of the receptor there exist two short regions of homology with the homoeotic proteins encoded by the *Drosophila* genes *Antennapedia* (*Antp*) and *fushi tarazu* (*ftz*)^{14,15} (Fig. 2). The first region of homology (upper comparison in Fig. 2) is in the homoeo box region and the second (lower comparison in Fig. 2) precedes the homoeo box by eight amino acids. Although the match is limited, these polypeptide regions were selected from a database of 300,000 residues as the most significant matches using a comparison window of 18 amino-acid residues. The homoeo box is a 180-base pair (bp)

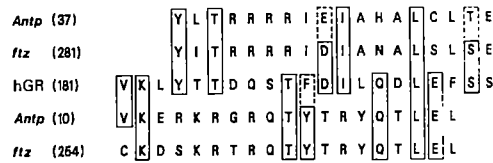


Fig. 2 Regional homology of the human glucocorticoid receptor with *Drosophila* homoeotic polypeptides. Numbers refer to the first amino-acid position in each protein segment of the *Antp* product¹⁴, the *ftz* product¹⁵, and hGR¹⁰. Broken lines indicate conservative amino-acid changes. Alignments were determined by a computer search of a database of 300,000 residues maintained by R. F. Doolittle, using an Inspect II program.

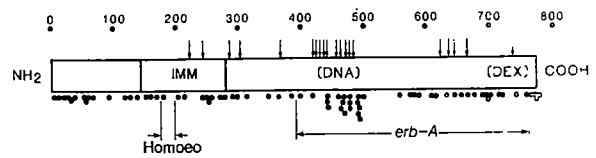


Fig. 3 Schematic representation of the primary amino-acid structure of the human glucocorticoid receptor, with the oncogene and homoeo box homologies indicated. The cysteine residues found in hGR¹⁰ are represented by arrows above the boxed coding region and basic amino acids (arginine and lysine) are denoted by solid circles. The immunogenic domain (IMM), determined from overlapping expression cDNA clones, the putative steroid-binding region (DEX) and DNA-binding domain (DNA) are indicated within the boxed receptor coding region. Numbers indicate amino-acid residues.

DNA sequence that is conserved in a variety of *Drosophila* homoeotic genes^{16,17}. These genes encode developmental regulatory proteins that appear to reside in the nucleus and thus, like the glucocorticoid receptor, appear to be *trans*-acting regulatory molecules^{18,19}. Whether these aspects of steroid receptor and homoeotic protein functions are reflected in structural relatedness awaits further investigation.

Structural analysis of the glucocorticoid receptor is required for understanding the mechanisms by which it regulates gene transcription. Here we attempt to construct a model for the hGR structure based on its primary amino-acid sequence¹⁰. Previous analyses of the rat receptor molecule have identified at least three domains which are physically separable by limited proteolytic cleavage²⁰⁻²². These include a major immunogenic domain, a steroid-binding region and a DNA-binding region. The immunogenic region resides on a proteolytic cleavage fragment of relative molecular mass (M_r) ~40,000 (40K), or 45% of the estimated receptor mass²⁰; the DNA- and steroid-binding domains are found on the remaining 55% of the molecule. Additional enzymatic cleavage studies localize the steroid-binding domain on a 22K peptide fragment containing no DNA-binding activity²³.

Expression cloning techniques^{10,24} were used to isolate three distinct but overlapping glucocorticoid receptor cDNA clones. The overlap between these clones indicates that boundaries for the immunogenic region occur between amino acids 145 and 278 (Fig. 3). Localizing the immunogenic domain at the amino terminus of the receptor indicates that the DNA-binding domain may be found in the carboxy-terminal half of the molecule. The steroid-binding region may be positioned more precisely by noting the properties of the variant human β -glucocorticoid receptor¹⁰. The β -hGR form differs from the α , or steroid-binding, receptor moiety in that the 50 carboxy-terminal amino acids of the α form are substituted by 15 amino acids in the β form¹⁰. As the *in vitro* translation product encoding the β -receptor fails to bind steroid¹⁰, the steroid-binding domain is localized at the extreme carboxy terminus.

Because the Cys-Lys-Arg-rich region (amino acids 421-481) embodies a particularly striking feature of the receptor and has

regions of homology with *v-erb-A*, we speculate that this region of the carboxy terminus may embrace the DNA-binding domain. It is interesting that the *Xenopus laevis* 5S RNA gene transcription factor TFIIIA²⁵⁻²⁷ contains a cysteine-rich region composed of nine repeated domains^{28,29}. The repeats are thought to be DNA-binding structures each containing a zinc ion at its centre. Although the hGR does not contain the large number of cysteines present in TFIIIA, the structural motif of a cysteine-rich region followed by basic amino acids is maintained. Accordingly, we propose a model for the receptor as shown in Fig. 3.

The two oncogenes *erb-A* and *erb-B*, encoded by AEV, are derived from cellular genes which comprise a multi-gene family in humans³⁰⁻³². The *erb-B* oncogene is a truncated form of the epidermal growth factor receptor and transforms erythroblasts, while *erb-A* strongly potentiates the effects of *erb-B* by blocking the maturation of erythroblasts at an early stage of differentiation³³⁻³⁸. Examination of the *v-erb-A* DNA sequence reveals that its amino terminus is synthesized as a fusion polypeptide with the viral *gag* product, while its carboxy-terminal 14 amino acids are derived from a combination of viral envelope and downstream *erb-B* sequences^{11,39}. Therefore, both the amino and carboxy termini of *v-erb-A* are apparently truncated relative to the cellular homologue.

The mechanism by which *v-erb-A* potentiates transformation is unknown, but the homology between *v-erb-A* and hGR suggests that the *erb-A* genes encode a family of transcriptional regulatory molecules. Assuming that the Cys-Lys-Arg-rich region of hGR represents the DNA-binding domain, then the region retained in *v-erb-A* might also bind specific DNA sequences. Thus, *v-erb-A* could exert its transformation effects by inappropriately stimulating gene expression in erythroblasts.

These results provide evidence for the relatedness of a family of proto-oncogenes to a known transcriptional regulatory protein, the glucocorticoid receptor. hGR also shows a discrete yet significant homology to the highly conserved amino acids in the homoeo box region of the *Drosophila* homoeotic genes *Antp* and *ftz*. This region is also found in the human genome, reinforcing the suggestion that the steroid receptor is composed of several functional domains. Localization of the immunogenic region in the hGR primary amino-acid sequence and the characterization of a variant receptor which does not bind steroid have permitted us to assign structural domains using experimental data gleaned from studies of the rat glucocorticoid receptor.

The unexpected indication from this study is that the steroid receptors and the *erb-A* proto-oncogenes share a primordial archetype. By analogy with the glucocorticoid receptor, these molecules may represent a new class of *trans*-acting factors that may be candidates for enhancer sequence binding proteins. Further characterization of the relationship of the glucocorticoid receptor to these oncogene products may elucidate the evolution of a family of eukaryotic transcriptional regulatory factors.

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Cloning of the breakpoint of an X;21 translocation associated with Duchenne muscular dystrophy

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Duchenne muscular dystrophy (DMD) is an X-linked recessive disorder which affects approximately 1 in 3,300 males, making it the most common of the neuromuscular dystrophies (see ref. 1 for review). The biochemical basis of the disease is unknown and as yet no effective treatment is available. A small number of females are also affected with the disease, and these have been found to carry X;autosome translocations^{2,3} involving variable autosomal sites but always with a breakpoint within band Xp21 of the X chromosome (implicated by other kinds of genetic evidence as the site of the DMD lesion⁴⁻⁶). In these female patients the normal X chromosome is preferentially inactivated, which it is assumed silences their one normal DMD gene, leading to expression of the disease. In one such affected female the autosomal breakpoint lies in the middle of the short arm of chromosome 21 (ref. 2), within a cluster of ribosomal RNA genes⁷. Here we have used rRNA sequences as probes to clone the region spanning the translocation breakpoint. A sequence derived from the X-chromosomal portion of the clone detects a restriction fragment length polymorphism (RFLP) which is closely linked to the DMD gene and uncovers chromosomal deletions in some male DMD patients.

The use of rDNA sequences as probes to identify the translocation junction was complicated by two factors. First, because the human genome contains 300-400 copies of the ribosomal gene distributed in 10 clusters on the five pairs of acrocentric chromosomes, the unique junction fragment would be difficult, if not impossible, to visualize on a Southern blot of total genomic

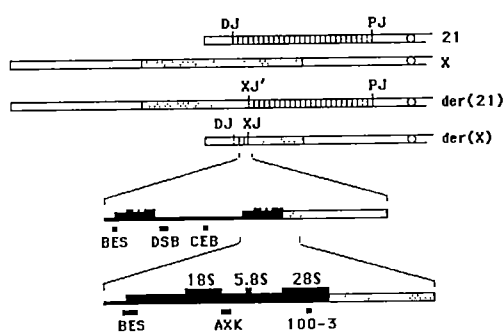


Fig. 1 Derivation of the translocation chromosomes der(X) and der(21). Below the der(X) chromosome is shown a schematic representation of the translocation chromosome at the breakpoint. The human ribosomal genes consist of a 43-kb unit repeated tandemly in blocks of 30–40 units on each of the 10 acrocentric chromosomes^{9,17}. The transcribed region is 13 kb long (represented as the solid black portion) and includes the 18S, 5.8S and 28S rRNA coding regions. The X-chromosomal portion of the translocation is represented by the stippled region, with the translocation breakpoint near the 3' end of the 28S gene. Below the schematic diagram are shown the subcloned regions BES, DSB, CEB, AXK and 100-3, used as probes.

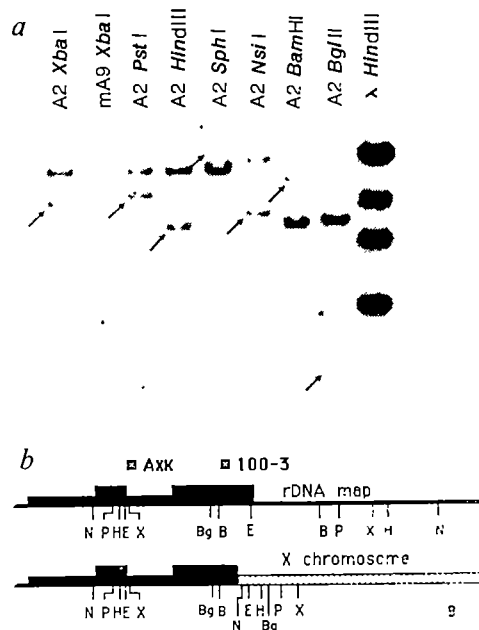


Fig. 2 Identification of the X;21 junction fragment by Southern blotting. **a**, DNAs were isolated¹⁸ from cultures of hybrid A2 and mouse A9, digested with the restriction enzymes indicated, separated by electrophoresis on a horizontal 0.6% agarose gel and transferred to nitrocellulose¹⁹. The human-specific rDNA subclone 100-3 was ³²P-radiolabelled by nick-translation¹⁹. Prehybridization of nitrocellulose filters was at 50 °C in 50% formamide, 5 × SSPE (1 × SSPE = 0.18 M NaCl, 0.01 M sodium phosphate pH 7.4, 0.001 M EDTA), 5 × Denhardt's, 150 µg ml⁻¹ sheared salmon sperm DNA, 0.1% SDS. Hybridization was in the same solution plus 10% dextran sulphate and 3 × 10⁶ c.p.m. ml⁻¹ of radiolabelled DNA. The filter was washed in 0.1% SDS and 0.2 × SSPE several times at room temperature and twice at 65 °C for 30 min each. Autoradiography was at -70 °C with an intensifying screen for 6 days. The arrows indicate unique human-specific bands derived from the translocation breakpoint. The major bands in each track indicate fragments derived from the 3–4 normal rDNA repeat units found on this chromosome. **b**, Restriction map of the normal rDNA repeat unit¹⁰ and the translocation junction. The map of the translocation junction was derived from Southern blot analysis of hybrid A2 DNA probed with either AXK or 100-3. The probe binding sites are indicated at the top of the figure. Restriction sites: B, BamHI; N, NsiI; E, EcoRI; H, HindIII; P, PstI; and X, XbaI.

DNA from the patient. Second, the ribosomal gene repeat unit is 43 kilobases (kb) long so that any rDNA sequence selected as a probe would have a 50% chance of being over 20 kb from the translocation junction—too far to be useful for screening Southern blots or λ libraries of 15–20-kb fragments.

To overcome the first problem, we separated the translocation chromosomes from the human acrocentric chromosomes by segregation in somatic cell hybrids generated by fusion of the patient's fibroblasts to mouse A9 cells. Three useful hybrids were generated. Hybrids A2 and F1 carried only the derivative X translocation chromosome, der(X), containing most of the X chromosome joined at band p21 to the distal end of the short arm of chromosome 21 (Fig. 1). This translocation chromosome has been shown by Southern blot analysis to carry 3–5 copies of the rDNA gene⁷. The third hybrid, C2-T10, carried the reciprocal translocation chromosome, der(21), that is, most of chromosome 21 joined at band p12 (the rDNA gene cluster) to the distal end of Xp. This chromosome was shown to carry 40–60 copies of the rDNA gene⁴. DNA from hybrid A2 [der(X)] was used to identify the junction fragment (by Southern blot hybridization) and to construct the library from which the junction fragment was isolated.

To overcome the problem of the long rDNA repeat unit, it was necessary to use as probes a series of human-specific rDNA sequences spaced over the rDNA repeat unit. Southern blot analysis of hybrid A2 cell DNA cut with a variety of restriction enzymes and hybridized with probes BES, DSB and CEB (Fig. 1) was carried out in a search for a unique restriction fragment derived from the X;21 junction region. A unique BamHI fragment was revealed by the BES probe, but the presence of a similar fragment in all human DNA samples suggested that it was derived from the natural junction at the distal end of the rDNA block of chromosome 21 (DJ in Fig. 1). Further studies confirmed this notion and allowed the ribosomal gene complex to be oriented as shown in Fig. 1, with transcription in the telomere to centromere direction (R.G.W. *et al.*, manuscript in preparation). The X;21 junction was not revealed by these probes, which suggests that the junction must be near the 3' end of the 28S gene, the only region not adequately covered by the three probes. To examine that region in more detail, a 600-base pair (bp) XbaI–KpnI fragment (AXK, Fig. 1) was isolated from the transcribed spacer region. This probe, when hybridized to XbaI, PstI and HindIII digests of hybrid A2 cell DNA, revealed, in addition to the expected rDNA bands, additional unique bands at an intensity corresponding to one copy per cell⁸. This result allowed a map of the junction region to be constructed

with putative XbaI, PstI and HindIII sites located in X-chromosomal DNA (Fig. 2).

Using this information, numerous attempts were made to clone the translocation junction using AXK as a probe. These experiments included construction of both phage and plasmid libraries from either partial digests of hybrid DNA or size-selected DNA fragments isolated by agarose gel electrophoresis. None of these cloning attempts yielded the junction fragment, forcing the conclusion that either ribosomal sequences or X-chromosomal sequences from this region were refractory to cloning. The former possibility seemed most likely as previous experiments had suggested that clones carrying the transcribed spacer region and 28S gene were underrepresented in total genomic libraries⁹. In order to isolate a human-specific probe much closer to the translocation breakpoint, the human 28S ribosomal gene sequence¹⁰ was compared with the homologous mouse sequence¹¹. Three human-specific regions were identified, subcloned and used to probe Southern blots of genomic DNA from the hybrid cell line. The results obtained for one of these probes (100-3) are shown in Fig. 2a. In addition to the restriction fragments previously identified with AXK, several new bands were visualized, including a 12-kb BamHI fragment containing the translocation breakpoint, which carried at most 1.8 kb of rDNA. To clone the junction fragment, DNA from the A2 hybrid line was cut with BamHI and size-selected fragments of 11–15 kb were cloned into λ Charon 35 (ref. 12).

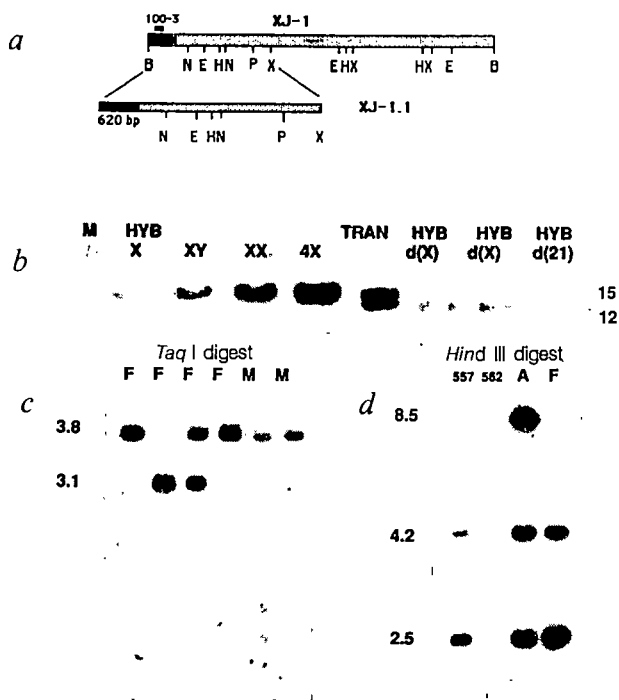


Fig. 3 *a*, Restriction map of the translocation junction clone XJ-1. The stippled area represents X-chromosomal material. The solid region represents that part derived from the rDNA cluster on chromosome 21. The translocation breakpoint is 620 bp from the 5' *Bam*HI site (unpublished data). The 100-3 probe binding site is indicated above the figure. The XJ-1.1 subclone from which the 1-kb *Nsi* fragment was derived is indicated below the map. The restriction site designation is described in Fig. 2. *b*, Localization of XJ-1 to the X chromosome. A Southern blot of total genomic DNA from several cell lines was probed with the 1-kb *Nsi*I fragment derived from XJ-1.1. The lanes contain 5 μ g of *Bam*HI-digested DNA from: M, mouse A9; HYBX, AHA 11a, an X-only mouse/human hybrid; XY, normal male; XX, normal female; 4X, a human female line with four X chromosomes; TRAN, the X;21 translocation patient; HYB d(X), the A2 and F1 hybrid lines carrying the der(X) chromosome; HYB d(21), the C2-T10 hybrid carrying the der(21) chromosome. The normal X chromosomes generate a 15-kb *Bam*HI fragment (lanes 2-5); the patient (lane 6) has a normal 15-kb band and a 12-kb translocation band; the hybrids carrying only the der(X) translocation chromosome (lanes 7, 8) have only the 12-kb band. *c*, A *Taq*I polymorphism associated with XJ-1.1. DNAs from four unrelated females (F) and two males (M) were digested with *Taq*I, electrophoresed, blotted and hybridized to the 1-kb *Nsi*I fragment from clone XJ-1.1 as described in Fig. 2 legend. The probe hybridizes to a 3.8- and a 3.1-kb fragment in females heterozygous for the RFLP (lane 3) and to either a 3.8- or a 3.1-kb band in homozygous females (lanes 1,2 and 4) and males (lanes 5, 6). *d*, Analysis of deletions for XJ-1.1 in males with DMD. DNAs from two unrelated males with DMD (557 and 562), the translocation patient (A) and her father (F) were digested with *Hind*III and probed with the *Nsi* fragment from XJ-1.1. Of the 50 DMD males tested in this manner, 49 had the normal banding pattern as seen with patient 557, that is, a 4.2- and a 2.5-kb band. One patient (562) showed no hybridizing sequences, indicating a deletion of this part of the chromosome. The translocation patient (A) shows a complex pattern consisting of a 4.2- and a 2.5-kb band derived from her normal X chromosome plus a 4.2- and an 8.5-kb band from her translocation chromosome. Her father (F) shows a normal banding pattern.

Approximately 10^6 recombinant phage were plated on *Escherichia coli* DB1161 (recA⁻recBC⁻sbcB⁻)¹³ and screened with 100-3, yielding five positive clones. Restriction mapping of these clones revealed all five to be identical. The map of the insert of one of these phage (XJ-1) is shown in Fig. 3a. The cloned fragment contained all of the restriction sites revealed by Southern blotting, consistent with its being the junction fragment. To further establish its identity as the junction fragment, a 1-kb *Nsi*I fragment, free of repeat sequences, was isolated from a *Bam*HI-*Xba*I subclone (XJ-1.1) and used to probe Southern blots of *Bam*HI-digested DNA from genomes with one, two or four human X chromosomes. The results (Fig. 3b) reveal a hybridization signal proportional to the number of human X chromosomes. This fragment also hybridized

Table 1 Linkage of XJ-1.1 to DMD

Family	Phase known		Phase unknown segregation
	Non-rec.	Rec.	
1			3:0
2			5:0
15	2	0	
17	1	0	
22			2:0
27			3:0
Total:	3	0	13

Rec., recombinant.

to a mouse-human hybrid carrying an X chromosome as the only human chromosome (Fig. 3b) and to one carrying only the short arm of the X chromosome (not shown). The hybridization pattern is also consistent with a translocation junction origin. In Fig. 3b, the *Nsi*I fragment of XJ-1.1 reveals a 15-kb *Bam*HI fragment from a normal X chromosome, a 12-kb band (that is, the cloned portion) from hybrid A2 with the der(X) translocation chromosome, and a double band from the patient's DNA, reflecting her two X chromosomes. A similar heterozygotic pattern in the patient's DNA was revealed with eight other restriction enzymes tested.

To show that the translocation junction is closely linked to the DMD locus, two studies were done. First, the *Nsi*I fragment of XJ-1.1 was used to seek RFLPs by probing Southern blots of DNA from four unrelated females cleaved with 27 restriction enzymes. A polymorphism detected with *Taq*I (Fig. 3c) was used to examine segregation patterns in several DMD families. Of 83 females in these families, 23 were heterozygous for the *Taq*I RFLP and no recombinants were found between XJ-1.1 and DMD in 16 meiotic events (Table 1). Of these 16 meiotic events there were 3 of known phase and 13 of unknown phase. All results were obtained with affected or normal sons of obligate DMD heterozygous carriers. A LOD score was calculated¹⁴ based on segregation of the *Taq*I RFLP in these DMD families and yielded a maximum of 3.4 at $\theta = 0$ centimorgans. The sample size of 16 meiotic events used in this analysis is quite small and many more informative families will be required to give a more precise estimate of linkage distance.

The second approach was to test a set of 50 DMD patients for deletion or rearrangement of the XJ-1.1 region. One of the 50 males (patient 562) displayed no hybridizing sequences (Fig. 3d), indicating deletion of the XJ-1.1 region. Recently, Kunkel *et al.*¹⁵ have isolated a probe (pERT87) closely linked to DMD which maps distal to XJ-1 and have detected deletions in 5 of 57 unselected DMD patients¹⁶. We have probed Southern blots (provided by Dr L. Kunkel) of DNA from all of these patients and found that all five also show deletions of XJ-1.1. Conversely, our panel of 50 DMD patients was screened by Kunkel for hybridization to pERT87. Only one patient, 562, failed to show hybridization to this probe (Kunkel, personal communication). The fact that these deletions are thought to be quite small (250-600 kb) suggests that XJ-1.1 and the probes described by Monaco *et al.*¹⁶ are closely linked to each other and to the DMD locus.

The underlying assumption in this work is that the translocation event disrupted the DMD locus, causing expression of the disease. Thus the junction clone or clones derived from chromosome walking should make it possible to find RNA transcripts from this region and eventually identify the gene product itself.

At a more immediate level, the translocation fragment will provide new RFLPs for use in carrier detection and prenatal diagnosis of DMD. This new RFLP will complement existing Xp21 probes and together with those described recently by Monaco *et al.*¹⁶ should provide enough closely linked markers to allow accurate diagnosis of most potential carriers.

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pERT87 probes, Southern blots of several DMD deletion patients and unpublished results from his laboratory; and Dr I. L. Andrulis for helpful discussions. Some of the earlier attempts at cloning the junction region involved significant effort on the part of Drs L.-C. Tsui, H. F. Willard and J. D. Freisen, whom we thank for their contributions. This work was supported by grants from the MRC of Canada (to R.G.W., P.N.R., and M.W.T.); the Muscular Dystrophy Association of Canada (to R.G.W., P.N.R. and M.W.T.); the Muscular Dystrophy Association, USA (to R.G.W.) and the NIH (to R.D.S.).

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An intron in the 23S ribosomal RNA gene of the archaeobacterium *Desulfurococcus mobilis*

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The archaeobacteria have been defined, at a molecular level, as constituting a third primary kingdom consisting of the methanogens, the extreme halophiles, and the sulphur-dependent extreme thermophiles. In reaching this conclusion, Woese and colleagues used the 16S ribosomal RNA as an approximate chronometer for evolutionary time and demonstrated that, at a nucleotide sequence level, the archaeobacteria are as different from the eubacteria and eukaryotes as the latter kingdoms are from one another^{1,2}. Current research on archaeobacteria is yielding valuable insights into the evolutionary relationships between archaeobacteria, eubacteria and eukaryotes, and into the early forms of cellular life³. Here, we extend this knowledge by providing the first evidence for the occurrence of an intron within any prokaryotic ribosomal RNA. The intron was found within the 23S rRNA gene of the sulphur-dependent and anaerobic Thermoproteale *Desulfurococcus mobilis*, which was isolated from hot acidic springs in Iceland at temperatures up to 97 °C^{4,5}. The intron contains 622 base pairs (bp); it is very A + T-rich (65%) compared with the 23S rRNA gene (34%), and it exhibits a large open reading frame. The splicing site occurs in domain IV of the 23S RNA at a position close to that of an intron of the lower eukaryote *Physarum polycephalum*⁶; the intron does not readily fall into one of the three classes of eukaryotic nuclear introns because it has features in common with those of classes I (rRNA) and III (transfer RNA)⁷.

23S rRNA genes from two archaeobacteria, the extreme thermophile *D. mobilis* and the extreme halophile *Halococcus morrhuae*, have recently been sequenced in this laboratory, and both exhibit sequence and secondary structural features that are

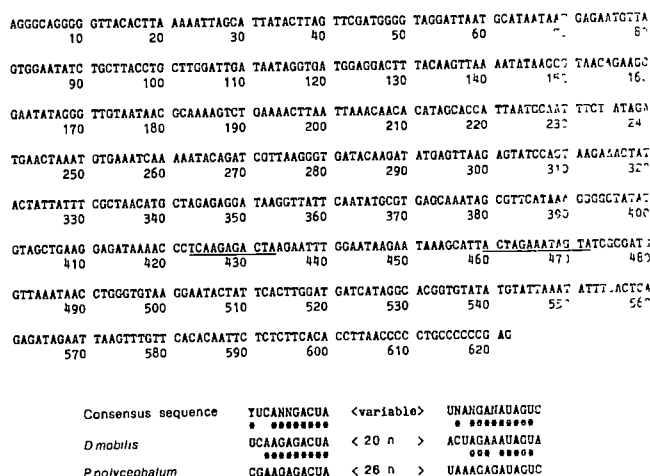


Fig. 1 Nucleotide sequence of the 622-bp intron, showing the strand corresponding to the transcribed RNA. The consensus sequences that may be required for the splicing of the eukaryotic rRNA introns are underlined in the sequence, and they are aligned below it (see text). The sequence was determined as follows. A 4-kilobase *Bam*HI fragment, subcloned into M13mp18, was digested with restriction enzymes specific for 4 bp. The DNA fragments were fractionated on a 4% polyacrylamide gel and cloned into M13mp18 or mp19 in both directions. Their directions were determined by 'figure 8' mapping²⁶. Single-stranded DNA was prepared and used as a template for DNA sequencing by the dideoxynucleotide method⁸, using [α -³²S]ATP (1,000 Ci mmol⁻¹; Amersham); the DNA was run on sequencing gels containing 4-6% polyacrylamide and salt or wedge-shaped gradients²⁷. Both DNA strands are sequenced, at least twice, and overlaps were obtained at each restriction site. Some differences were observed between our restriction map of the 23S rRNA gene and that published by Neumann *et al.*⁹. Some of the restriction enzyme cuts that we found were reported by Neumann *et al.* only for the closely related *D. mucosus* rRNA operon, and our estimate of the 16S-23S RNA spacer region was larger. However, we agree that the 5S RNA and 23S RNA genes are uncoupled, which is an exclusive property of the *D. mobilis* operon⁹.

exclusive to the archaeobacteria (H. Leffers, J.K. and R.A.G., unpublished work). These sequences were aligned with those of other eubacterial and eukaryotic 23S-like rRNA genes and the *D. mobilis* gene was shown to contain a large 622-bp insert. Its nucleotide sequence is given in Fig. 1.

To establish whether the insert was, indeed, an intron, the 23S RNA was sequenced over the putative splicing site using reverse transcriptase. A 5' end-labelled restriction fragment was used as a primer with purified 23S RNA as the template; sequences were obtained using dideoxynucleotides⁸. Figure 2 shows an alignment of the resulting complementary DNA sequence with that of the 23S RNA gene. The result demonstrates that the insert was excised from the pre-rRNA and thereby establishes both the nature and location of the intron.

Southern hybridization studies between the purified large rRNAs and total chromosomal DNA confirmed the result of Neumann *et al.*⁹ that only one operon exists for the large rRNAs (data not shown). There can be little doubt, therefore, that this gene is both transcribed and spliced. This view, that it is not a pseudogene, was reinforced by demonstrating that 300 nucleotides of cDNA, sequenced around the splicing site (Fig. 2) and the 5' end of the 23S RNA, were identical to the gene sequence.

The intron is located within the functionally important domain IV of the 23S-like RNAs¹⁰ (Fig. 3). The sequence shown is that of *D. mobilis*, while the numbering system and secondary structure are based on *Escherichia coli* 23S RNA¹¹. The uncertainty in the assignment of the splicing site, at nucleotide 1,953 (± 1), is due to an A-G direct repeat occurring at the exon-intron junctions. The insert is flanked by highly conserved sequences that contain several post-transcriptionally modified nucleotides in *E. coli*¹⁰ (Fig. 3) and at least one in *D. mobilis* 23S RNA

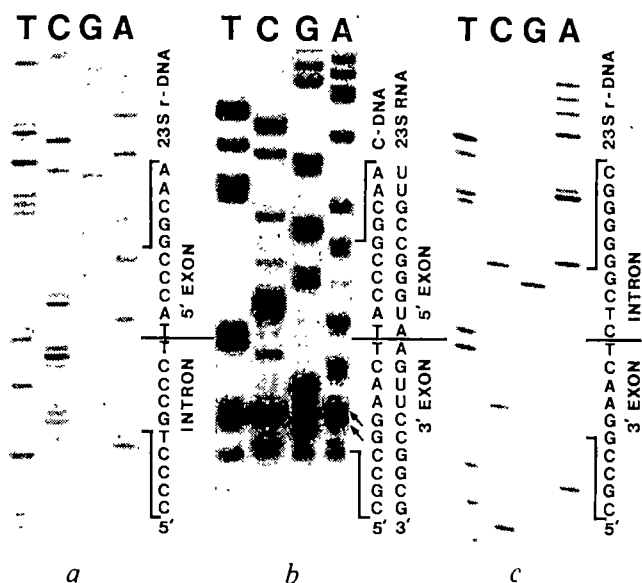


Fig. 2 Gel *b*, sequencing gel showing the sequence of cDNA obtained through the splicing site using reverse transcriptase. This gel is flanked by DNA sequencing gels showing the 5' (*a*) and 3' exon-intron junctions (*c*). Over 200 nucleotides of the cDNA sequence were read and they corresponded exactly to the gene sequence.

Methods. The gene sequence was determined by hybridizing 3 pmol of a double-stranded *HhaI*-*HaeIII* restriction fragment, which had been 5' end-labelled using [γ - 32 P]ATP (6,000 Ci mmol $^{-1}$; ICN Pharmaceuticals), to 5 pmol 23S RNA. The hybridization reaction was performed in 15 μ l buffer (20 mM HEPES pH 7.0, 0.4 M NaCl, 1 mM EDTA, 89% formamide) by heating at 95 $^{\circ}$ C for 2 min, then at 55 $^{\circ}$ C for 90 min, and cooling quickly. Sequencing reactions were performed in 10 μ l buffer (50 mM Tris-HCl pH 8.3, 10 mM MgCl $_2$ and 2 mM dithiothreitol) containing 200 μ M dNTP, 200 μ M of the appropriate dideoxy dNTP, 0.5 pmol primer template complex and 10 units AMV reverse transcriptase (Life Sciences, Florida). cDNA was fractionated on 6% polyacrylamide gels (20 cm \times 40 cm \times 0.02 cm). Strong termination of nonspecific nature was detected in the cDNA track at G 1,960; this may result from a modification of G 1,959.

(Fig. 2). Chemical modification studies have also indicated that part of this region is accessible on ribosomes of both eubacteria and eukaryotes 11 . Each of these characteristics (sequence conservation, post transcriptional modification and location on the ribosomal surface) is a strong indicator of a functionally important region of RNA, although no definite function has yet been established 10 .

It appears to be a general property of rRNA (and tRNA) introns that they are clustered in functionally important regions. Two rRNA introns have been located in neighbouring positions of domain IV: one occurs in the *P. polycephalum* gene at position 1,949, which is 3–5 nucleotides 5' of the present intron 6 , and another occurs in *Tetrahymena pigmentosa* at position 1,925 (ref. 12) (Fig. 3). Most of the others have been located in a region of domain V that has been strongly implicated in the peptidyl transfer reaction 11 . The function(s) of the introns remains unclear. The splicing reaction could be a mechanism for activating ribosomes after assembly 13 ; it could also constitute a proofreading for the correct assembly of important functional sites.

Eukaryotic nuclear introns have been divided into three groups on the basis of their splicing mechanisms: (1) rRNA; (II) mRNA and (III) tRNA 7 . In order to classify the present intron, we aligned its sequence with the 501-nucleotide intron that occurs at an adjacent position in the 23S-like rDNA of *P. polycephalum* 6 . Two common sequences were detected at similar relative positions (Fig. 1). They correspond to the consensus sequences of class I introns that are essential for correct

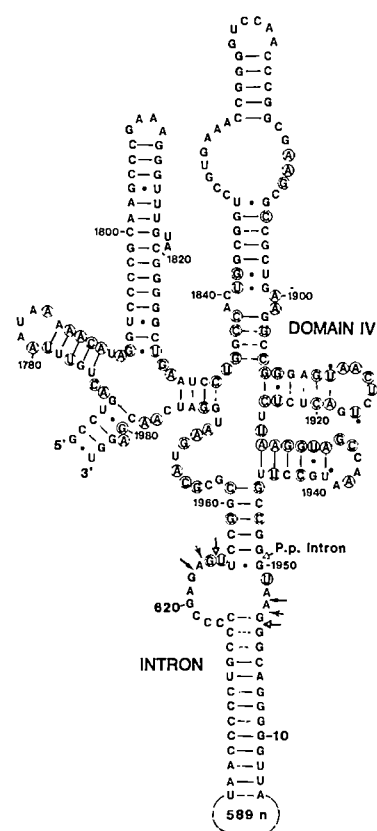


Fig. 3 Secondary structure of a major part of domain IV of the pre-23S RNA of *D. mobilis*. The numbering system and the secondary structure derive from that of *E. coli* 23S RNA 11 . The ringed nucleotides are highly conserved in 12 sequenced 23S-like RNAs from eubacteria, archaeobacteria, chloroplasts and the eukaryotic cytoplasm 10 . Domain IV of *E. coli* 23S RNA 11 contains several modified nucleotides and these are indicated by asterisks. *D. mobilis* exhibits at least one modification, at G 1,959 (see Fig. 2). The three pairs of arrows indicate the three possible splicing junctions, and the putative helix at the start of the intron is shown. P.p. intron, the position of the *P. polycephalum* intron 2 (ref. 6).

splicing $^{14-16}$. Other properties, however, are not shared by class I introns; these include the putative base pairing between the ends of the RNA (Fig. 3), the lack of internal core structure, and the absence of a uridine at the 3' end of the 5' exon 14,15,17 . The latter are characteristics of class III introns that occur in the anticodon loops of nuclear tRNAs; these exhibit no obvious consensus sequences and the splicing reaction is probably directed by an endonuclease recognizing invariant features of the tRNA structure 18,19 .

Large open reading frames (ORFs) are common to the transcribed introns of many mitochondrial mRNAs, some large rRNAs and chloroplast tRNAs 15,20 . As illustrated in Fig. 4, the intron in *D. mobilis* also contains one ORF that starts 57(\pm 1) nucleotides from the 5' exon-intron junction, continues through



Fig. 4 The locations of stop codons in the three reading frames of the RNA that is transcribed from the intron and the flanking region of the 23S RNA gene. ORF denotes the open reading frame. The plot was obtained using a Staden computer program 28 .

the 3' junction and extends 215 (± 1) nucleotides into the 3' exon. The likelihood that it codes for a protein is reinforced by the fact that the other frames of the intron contain 28 and 30 stop codons. Moreover, two putative Shine-Dalgarno sequences (5' AGGA 3' and 5' GGGGT 3') precede the ORF; these lie 3 and 7 nucleotides, respectively, from the start codon and both can interact with the sequence 5' ACCTCCT 3' at the 3' end of the 16S RNA (data not shown). Furthermore, a 30-nucleotide region preceding these putative Shine-Dalgarno sequences is very A+T-rich (80%); this feature is common to ORFs of introns in the rRNA genes of *Neurospora crassa*²¹. The extension of the ORF by 215 (± 1) nucleotides into the 3' exon suggests either that it is translated prior to splicing or that the RNA circularizes after splicing, as was found for intron II of *T. pigmentosa*²², such that translation is terminated by stop codons upstream from the initiation codon.

In conclusion, this is the first intron to be found in a rRNA gene of any prokaryotic system. The result complements those introns found in archaeobacterial tRNAs, that is, the small putative introns in tRNA^{Leu} and tRNA^{Ser} of *Sulfolobus solfataricus*²³ and a 105-bp intron in the tRNA^{Trp} gene of *Halobacterium volcanii*²⁴; all exhibit important differences compared with their eukaryotic counterparts. Collectively, though, these results reinforce the view that the archaeobacteria, in general, share more characteristics with the eukaryotes than do the eubacteria, where an intron has been reported only for the thymidylate synthase gene of the phage T4²⁵.

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Near-reciprocal phenotypes caused by inactivation or indiscriminate expression of the *Drosophila* segmentation gene *ftz*

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Early in development, *Drosophila* embryos express the segmentation gene *fushi tarazu* (*ftz*)^{1,2} in a 'zebra' pattern of active and inactive stripes, each about the width of a segment primordium³. If the *ftz* gene is prevented from functioning, alternating portions of the body normally derived from the active stripes fail to develop, resulting in larvae which lack the denticle bands normally formed by the mesothorax and odd-numbered abdominal segments (that is, thoracic segment T2 and abdominal segments A1, A3, A5 and A7). Here, using the *Drosophila* heat shock protein 70 (*hsp70*) gene promoter⁴⁻⁷ to drive widespread expression of *ftz* transcripts on heat shock, I find that unrestricted *ftz* activity can cause a reciprocal 'pair-rule' phenotype—that is, the absence of the denticle bands which are normally derived from segments T1, T3, A2, A4, A6 and A8. These results show that both the 'on' and 'off' states of *ftz* gene expression have instructive roles in the development of alternating regions of the body, and hence suggest that the *ftz* gene acts combinatorially with other pair-rule genes (for example, *even-skipped*, *odd-skipped*, *paired*)⁸⁻¹⁰ to establish the metameric pattern of the body.

To examine the consequences of indiscriminate *ftz* expression, a sequence coding for most of the mature *ftz* messenger RNA (including the entire open reading frame of the *ftz* protein)¹¹ was fused to the promoter of the *hsp70* gene⁵⁻⁷, and this hybrid gene was incorporated into the germ line by P-element-mediated transformation^{12,13} (see Fig. 1). Four independent transformant lines were obtained: two, called HSF2 and HSF3, have been examined in detail. The HSF2 and HSF3 transformants result from insertions of a single copy of the *P(hsp70-ftz, Adh⁺)*

element into chromosomes 2 and 3, respectively (segregation and Southern blotting data not shown): in both cases, animals homozygous for the transduced element are viable and fertile.

The *hsp70* promoter can be induced by heat shock throughout the life cycle except for the first few hours of embryogenesis (up to the blastoderm stage, ~2.5-3.5 h after egg laying, 25 °C) and the late stages of oogenesis^{5-7,14}. Both HSF transformants expressed the *hsp70-ftz* hybrid transcript in response to heat shock during the blastoderm stage as well as in older embryos (see, for example, Fig. 2). When HSF embryos were heat-shocked during the blastoderm stage, approximately half of the resulting larvae displayed pair-rule phenotypes (described below and in Fig. 3). Similar segmentation phenotypes were not observed when HSF embryos were exposed to heat shock after the blastoderm stage, nor were they observed when control embryos lacking the *hsp70-ftz* hybrid gene were heat-shocked at any time during embryogenesis.

Previous studies have shown that almost all cells of the body induce the *hsp70* promoter on heat shock⁵⁻⁷. Because wild-type embryos normally display a tightly restricted zebra pattern of *ftz* transcripts during the blastoderm stage, the altered segment patterns caused by heat-shocking HSF embryos during this period probably result from ectopic expression of the *hsp70-ftz* transcripts.

Before describing the HSF phenotypes, it is helpful to consider the pair-rule phenotype caused by apparent null mutations in the *ftz* gene. *ftz*⁻ embryos develop into larvae which appear to delete every other segment^{1,2} (that is, segments T2, A1, A3, A5 and A7; see Fig. 3*b, c*). This assessment is based primarily on the presence or absence of conspicuous belts of ventral hairs (denticle bands) which are formed by anterior portions of each segment. However, examination of sensory organs such as Keilin's organs, which are positioned at or near the anteroposterior compartment boundary¹⁵, as well as the patterns of dorsal hairs, which display segment-specific differences in both anterior and posterior compartments of several segments^{15,16}, indicates that the deleted regions are composed of segment-length domains which begin and end near the anteroposterior compartment boundaries within adjacent segments (Fig. 3; see also refs 9, 10). Note that the regions deleted

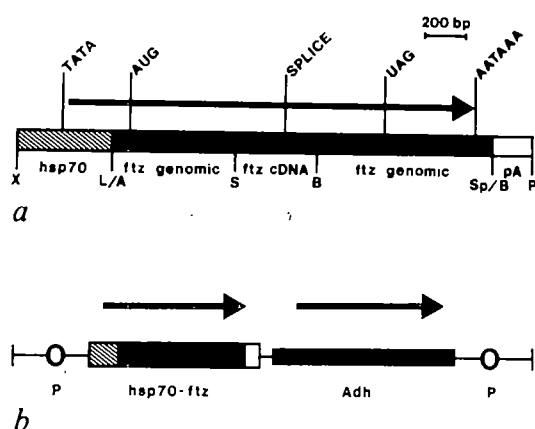


Fig. 1 Composition of the *hsp70-ftz* fusion gene (a) and generation of germline transformants (b). a, The fusion gene is composed of three pieces: (1) a 450-base pair (bp) sequence containing the *hsp70* promoter (hatched bar), (2) a composite *ftz* gene which contains the uninterrupted open reading frame present in the mature *ftz* transcript (solid bar), and (3) a 150-bp fragment of the 3' end of a *Xenopus* β -globin complementary DNA including a 23-bp poly(A) tail (open bar). The *hsp70* (132E3) and globin fragments are described in refs 33 and 34; the composite *ftz* gene was generated by substituting a 580-bp region of the genomic clone *Dm437* (ref. 35) containing the single *ftz* intervening sequence, with the corresponding 400-bp region of the cDNA clone *G20*⁺ (ref. 11) as shown (X, A, S, B, Sp and P represent, respectively, the *Xba*I, *Ava*II, *Sal*I, *Bgl*II, *Sph*I and *Pst*I sites; indicates that the fragments are joined by blunt-end ligation) (see ref. 11 for the *ftz* cDNA and genomic sequences). The 5' portion of the fusion gene transcript (arrow) consists of 200 bp of the 5'-untranslated *hsp70* transcript joined by a 10-bp linker sequence (L = AAGCTTGGGC) to the *ftz* gene about 80 bp in front of the start of the major open reading frame (AUG). The 3' end of the fusion gene, beginning at the end of the open reading frame (UAG), consists of ~400 bp of the *ftz* 3'-untranslated region, the putative polyadenylation signal (AATAAA), and the next 100 bp of the *ftz* genomic sequence. b, The *hsp70-ftz* fusion gene was inserted into the P-element transformation vector pPA-1 (J. Posakony, unpublished) which carries the *Adh* (alcohol dehydrogenase) gene as a selectable marker. The directions of transcription of the *hsp70-ftz* and *Adh* genes are indicated by arrows; P, sites for P-factor-mediated integration. Germline transformants were then generated in *Adh*^{fr6} *cn*; *ry* hosts by standard means^{12,13}. The same hosts were used as untransformed controls for the heat-shock experiments.

in *ftz*⁻ embryos cannot be precisely demarcated, partly because of the lack of sufficient cuticular landmarks, but also because they are somewhat variable in extent (see Fig. 3 legend). Also, a small proportion of *ftz*⁻ embryos (usually <20%) show more extreme segmental fusions in which the denticle bands associated with one or more additional segments appear to be partially or completely deleted.

In contrast to *ftz*⁻ embryos, HSF2 and HSF3 embryos heat-shocked during the blastoderm stage can give rise to abnormal larvae which partially or completely lack the denticle bands normally formed by segments T1, T3, A2, A4, A6 and A8—exactly those denticle bands which are retained in *ftz*⁻ embryos (see, for example, Fig. 3). Thus, conditional, and presumably unrestricted, expression of the *hsp70-ftz* hybrid gene can cause a pair-rule phenotype which is superficially reciprocal to that resulting from absence of the *ftz* gene.

Both the frequency and extents of these reciprocal phenotypes are variable, perhaps because an extreme phenotype results only when the embryos are heat-shocked during a brief, optimal period during the blastoderm stage (for example, late enough for the *hsp70* promoter to be fully inducible, but early enough to allow sufficient *ftz* gene product to accumulate before it has to act). This variability has the fortunate consequence that a broad range of phenotypes can be examined. As in the case of

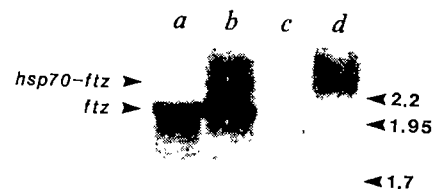


Fig. 2 Heat-shock control of the *hsp70-ftz* fusion transcript. The presence of normal *ftz* and hybrid *hsp70-ftz* transcripts was assayed by Northern blot analysis using a probe complementary to the composite *ftz* gene (solid bar in Fig. 1a). a, HSF2 embryos aged 2 3/4–3 1/4 h after egg laying express the normal *ftz* transcript (~2.0 kilobases (kb))^{11,35,36} and, at a low concentration, the 2.2-kb hybrid *hsp70-ftz* transcript (not visible in this exposure). b, HSF2 embryos of a similar age to those in a, but heat-shocked for 20 min, express the 2.0-kb *ftz* transcript as well as a series of larger transcripts, including a discrete 2.2-kb transcript: the 2.2-kb transcript almost certainly corresponds to the *hsp70-ftz* transcript (which should be ~200 bp longer than the normal *ftz* transcript if terminated near the putative polyadenylation signal), and the larger transcripts may correspond to hybrid transcripts which terminate farther downstream. Note that the *hsp70-ftz* transcripts are less abundant than the native *ftz* transcripts; this difference may be due to poor induction of the fusion gene in some embryos (consistent with the finding that only about half of the HSF embryos heat-shocked during this period give rise to larvae showing reciprocal *ftz* phenotypes). It may also indicate that the levels of ectopic *hsp70-ftz* transcripts necessary to alter the segment pattern may be lower than the levels of *ftz* transcripts normally expressed in the zebra pattern. c, Control embryos (6–10 h after egg laying) heat-shocked for 20 min express low levels of the normal *ftz* transcript. d, HSF2 embryos of a similar age to those in c, but heat-shocked, express low levels of the normal *ftz* transcript as well as high levels of the fusion transcripts.

Methods. Embryos were grown at 25 °C and heat-shocked at 35 °C for 20 min. Northern blot analysis was performed as described previously³⁷ (~8–10 μ g of total nucleic acid was loaded per lane). The *ftz* probe was generated by gel extracting and then nick-translating of the composite *ftz* gene. Neighbouring lanes loaded with RNA from 2 3/4–3 1/4-h-old HSF2 embryos were probed with sequences complementary to the major actin transcripts (1.7, 1.95 and 2.2 kb)³⁸, providing size markers for the *ftz* and *hsp70-ftz* transcripts. Similar results were obtained using HSF3 embryos. Low levels of an additional 1.75-kb transcript were detected in all four lanes; whether this represents a *bona fide ftz* transcript is unknown.

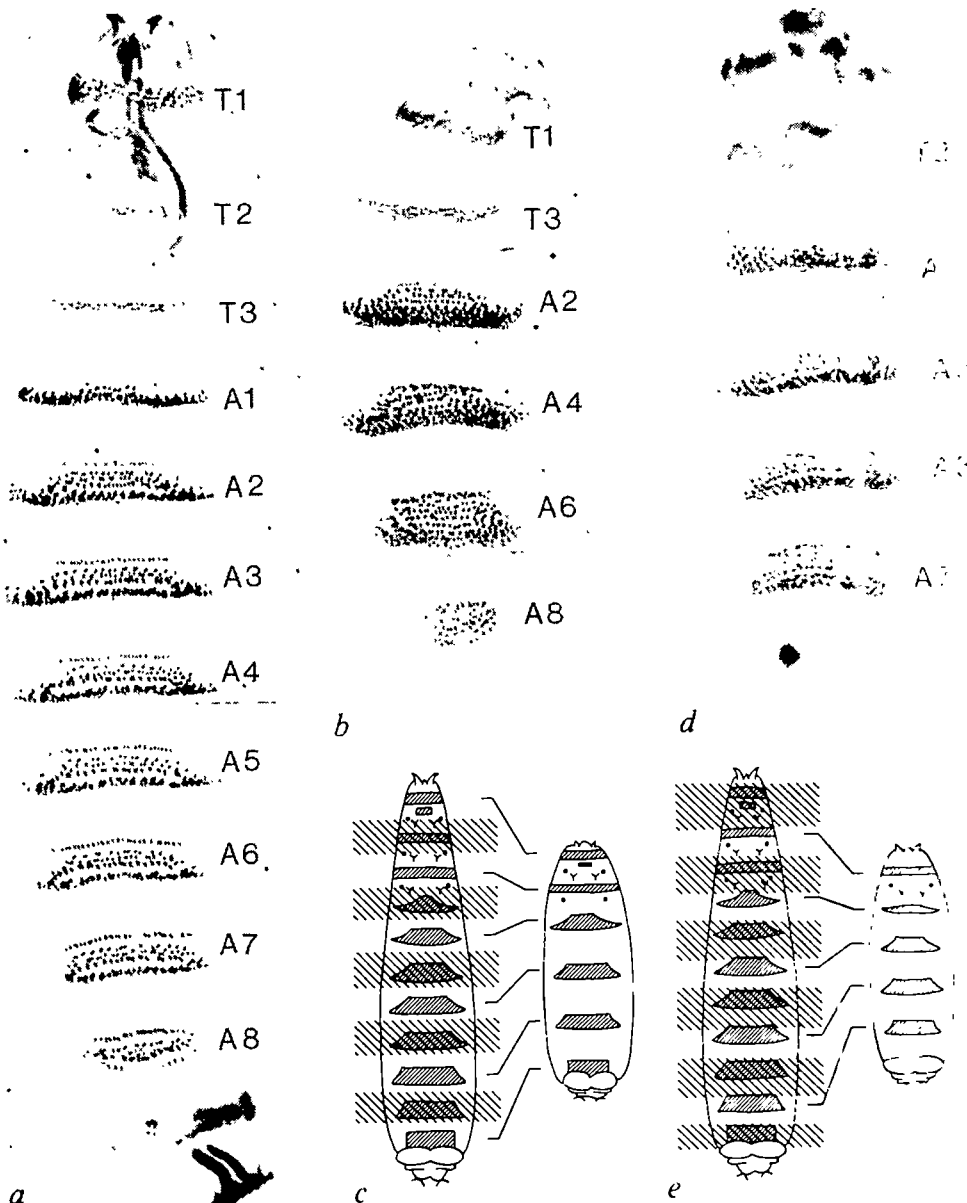
ftz⁻ embryos, there seems to be no absolute boundary between deleted and retained regions; however, the deletion pattern shown in Fig. 3e illustrates the boundaries which are usually respected. Note that the deletion patterns of *ftz*⁻ and heat-shocked HSF larvae are not perfectly reciprocal, but appear to overlap to some extent (Fig. 4).

The *ftz* gene is one of about 10 'pair-rule' genes that, when mutant, cause the apparent deletion or fusion of homologous portions of every other segment^{8–10}. Moreover, each of these genes is associated with a particular deletion pattern, suggesting that they are responsible for the development of overlapping, but different, repeating intervals of the segment pattern^{8–10}. The spatial distributions of transcripts of two pair-rule genes, *ftz* and *hairy* (*h*), have been determined; both are expressed in zebra patterns in which the active stripes correspond approximately to the regions of the body that are deleted in mutant embryos^{3,17}. The emerging picture therefore seems to be that the deletion patterns associated with the different pair-rule genes define alternating regions of the body where these genes are both expressed and required for normal development. Why then are the *ftz* and *h* genes (and possibly the remaining pair-rule genes) silent in the reciprocal portions of the body where their activities seem to be dispensable?

The results presented here argue strongly that the *ftz* gene is

Fig. 3 Near-reciprocal pair-rule phenotypes of *ftz*⁻ and heat-shocked HSF embryos. **a**, Wild-type first-instar larva. The three thoracic and first eight abdominal segments (T1-T3, A1-A8) each bear a characteristic band of ventral hairs (denticles) formed by the anterior compartment, **b**, **c**, *ftz*⁻ larva (homozygous for an apparent null allele, *ftz*⁹⁰⁹³; ref. 39). As described in the text, mutant larvae form only half the number of denticle bands (those belonging to segments T1, T3, A2, A4, A6 and A8)^{1,2} and hence appear to lack every other segment. In fact, the deleted regions are not segments, but rather segment-length units which begin close to the anteroposterior compartment boundary in one segment and end close to the boundary within the next segment. This is particularly clear in the thoracic segments because: (1) the patterns of dorsal and ventral hairs in each 'double segment' are composites of the anterior (a) and posterior (p) portions of adjacent segments (that is, T1a+T2p, T3a+A1p, A2a+A3p, etc.), (2) two sets of lateral sensory hairs⁴⁰ are often formed in each 'double segment' (normally each segment has a single set positioned just anterior to the compartment boundary within the segment), suggesting that a region slightly less than a segment's length lying between the lateral hairs in adjacent segments has been deleted, and (3) partial or complete Keilin's organs (which normally lie on or near the anteroposterior compartment boundary in each thoracic segment¹⁷) are usually present in the T1aT2p double segment, but are present only rarely in the T3aA1p double segment (deletion of a segment-length unit beginning and ending around the compartment boundaries within adjacent segments might be expected to leave behind partial or complete Keilin's organs more frequently when both segments are thoracic than when one is thoracic and the other abdominal). The exact boundaries between deleted and retained portions of the body seem to vary somewhat. For example, Keilin's organs are composed of three sensory hairs, two derived from the anterior compartment and a third derived from the posterior compartment¹⁵. In the T1aT2p double segment of *ftz*⁻ embryos, the Keilin's organs are sometimes rudimentary di-hairs or mono-hairs, or are absent. A further aspect of the *ftz*⁻ phenotypes is that some pattern elements, such as specific sensory hairs or rows of denticles, appear to be duplicated in each metameric unit¹. One consequence of this phenomenon, which has also been observed with mutations in other pair-rule genes¹⁸, is that the denticle bands appear broader than in wild-type embryos. **d**, **e**, Heat-shocked HSF3 larva. In contrast to *ftz*⁻ larvae, HSF larvae derived from embryos heat-shocked during the blastoderm stage often partially or completely lack the denticle bands associated with segments T1, T3, A2, A4, A6 and A8. Partial deletions often appear as fusions which join the denticle bands of particular pairs of segments (T1+T2, T3+A1, A2+A3, etc.). In progressively more extreme deletions, the denticle bands belonging to the more anterior segments (T1, T3, A2, etc.) appear progressively narrower or are eliminated, leaving the denticle band derived from the more posterior segments (T2, A1, A3 etc.) largely intact (as in **d**). Thus, the deleted regions seem to be slightly larger than segment-length units which begin and end just outside the boundaries of segments T1, T3, A2, A4, A6 and A8. As in the case of *ftz*⁻ embryos, the limits of the deletion pattern illustrated in **e** seem to define the usual stopping line, but some embryos show more extreme deletion phenotypes. Because of the variation in phenotype, these limits should be considered provisional. **a**, **b**, **d**, Dark-field photomicrographs (ventral aspects, positive images; $\times 120$). **c**, **e**, The regions deleted from the normal pattern (after ref. 8). The denticle bands are shaded and the missing regions indicated by hatched bars; Keilin's organs and ventral pits are shown as Y and o symbols, respectively. Keilin's organs are found only rarely in the T3aA1p double segment of *ftz*⁻ larvae and are therefore not indicated in this position in **c**. No attempt has been made to map the deleted regions anterior to T1 or posterior to the A8 denticle band, though some portions of these terminal regions fail to develop in each case.

Methods. **d**, **e**, Embryos were collected and allowed to develop at 25°C and were usually heat-shocked for 20 min at 35°C, although shocks of 5 or 10 min were also effective at generating reciprocal *ftz* phenotypes. Typically, 500-1,000 fertilized eggs were collected during a 30-min period and shocked between 2 and 3 h after egg laying. In these conditions, ~50% of the resulting larvae showed partial or complete deletions of the denticle bands of at least three alternating segments; fewer than 1% of the embryos heat-shocked between 3 $\frac{1}{4}$ and 3 $\frac{3}{4}$ h after egg laying (or at any time thereafter) developed into larvae showing reciprocal *ftz* phenotypes. HSF2 and HSF3 embryos responded similarly to heat shock.



normally silent in the 'off' stripes because it must be off to allow these regions to develop. Thus, the alternating on and off stripes of *ftz* activity seem to be equally critical, in their respective domains, for organizing the metameric pattern of the body. Similar evidence is not yet available for the *h* gene. However, dosage studies of another pair-rule gene, *run*^{8,18}, have shown that extra wild-type copies can cause pair-rule deletions which are nearly reciprocal to the deletions resulting from loss of gene function (ref. 10 and J. P. Gergen and E. Wieschaus, personal communication). Though the interpretation is less clear in this

case, it is possible that these 'anti-run' phenotypes are caused by elevated levels of *run* expression in regions where the gene must normally be either silent or expressed at a low level.

These results can be explained by positing that the pair-rule genes act combinatorially to initiate the development of repeating portions of the segment pattern (see also ref. 10). For example, each cell along the anteroposterior axis of the blastoderm may express a specific combination of pair-rule genes (perhaps in response to a periodic spatial cue). This combination of genes then initiates the development of a specific interval of

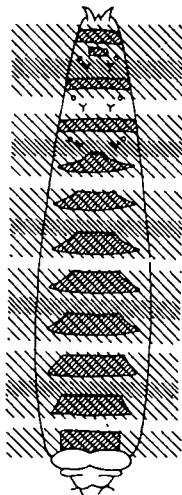


Fig. 4 Overlap between the *ftz*⁻ and heat-shocked HSF pair-rule phenotypes. The regions of the body deleted in heat-shocked HSF larvae (Fig. 3e) are largely anterior to the regions deleted in *ftz*⁻ larvae (Fig. 3c). Note that the deletion patterns are not perfectly reciprocal: regions of common overlap (heavy shading) and common exclusion (unshaded) are found in alternating intervals along the body.

the repeat pattern, perhaps by activating the correct patterns of expression of other genes such as *engrailed*^{8,19-22} and the segment polarity genes^{8,9} which function subsequently to control growth and cell pattern within segments. Accordingly, both the on and off states of pair-rule gene function would have equal instructive roles because the developmental behaviour of any given cell or region would depend on the initial code-word of on and off pair-rule genes. In this regard, pair-rule genes may be acting in a manner functionally analogous to that of homoeotic genes of the bithorax and *Antennapedia* complexes²³⁻³¹.

Though this interpretation is prompted in part by the reciprocal nature of the *ftz*⁻ and heat-shocked HSF phenotypes, it should be noted, as shown in Fig. 4, that these phenotypes are not perfectly reciprocal: some regions of the body are deleted in both *ftz*⁻ and heat-shocked HSF larvae while other regions appear unaffected in either case. Regions of overlap between the two deletion patterns suggest that *ftz* and perhaps other pair-rule genes do not function simply as binary switches, but rather that differences in relative levels of their gene products, or perhaps temporal sequences of alternating expression, may also be important in defining specific portions of the segment pattern. Note that the stripes of cells expressing *ftz* transcripts narrow towards the end of the blastoderm stage^{3,32}, indicating that the gene may be on to different extents, or first on and then off, in some cells of each stripe.

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Simian virus 40-mediated *cis* induction of the *Xenopus* β -globin DNase I hypersensitive site

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Regions in chromatin which are hypersensitive to the action of DNase I appear to be associated with sites of genetic activity; the association between DNase I hypersensitivity and transcriptional activation is well known¹. In the case of the chicken β -globin gene the establishment of a DNase I hypersensitive site is dependent on tissue-specific *trans*-acting factors^{2,3}. Such factors have also been implicated in the action of viral and cellular enhancers⁴⁻¹⁰, which are themselves hypersensitive to DNase I¹¹⁻¹⁴. Enhancers have been defined operationally as DNA sequences which act in *cis* to potentiate transcription from their own, heterologous or cryptic promoters. This activity is essentially unaffected by changes in the orientation, position (5' or 3') or distance of the enhancer element with respect to its cognate promoter (ref. 15 for review). We demonstrate here that the transcriptional rescue of the *Xenopus laevis* β -globin gene by simian virus 40 (SV40) sequences including the enhancer coincides with the conferment of DNase I hypersensitivity upon that gene, and that this occurs in the absence of any change in the complement of *trans*-acting factors. These results suggest that a propensity to form sites hypersensitive to the action of DNase I is encoded in the primary sequence of DNA¹⁶, and that this predilection is aggravated by SV40 sequences, perhaps through a mechanism dependent on supercoiling.

Neither human nor rabbit β -globin genes are expressed when they are introduced by transfection into HeLa cells on plasmid vectors in a transient assay, but transcription of these genes is restored by linkage in *cis* to the SV40 enhancer^{17,18}. We have examined the behaviour of the *X. laevis* β_1 -globin gene in similar conditions. HeLa cells were transfected with recombinant plasmids and left for 48 h, after which both nuclei and total cytoplasmic RNA were prepared. The RNA was analysed for the presence of correctly initiated globin transcripts by quantitative S₁

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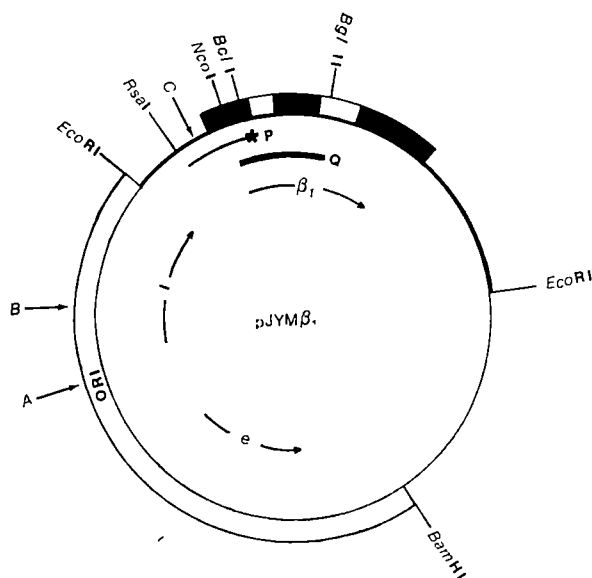


Fig. 1 The SV40-*Xenopus* β_1 -globin gene construct, pJYM β_1 . pXG β_1 (ref. 28) contains a 3.5-kilobase *EcoRI* *X. laevis* β_1 -globin gene fragment cloned into the *EcoRI* site of pAT153 (ref. 29). pJYM Δ RB was made by ligating the large *EcoRI*/*Bam*HI fragment containing all the early and part of the late transcription units of SV40 to an *EcoRI*/*Bam*HI fragment from pBR322 from which sequences poisoning replication in eukaryotic cells had been deleted³⁰. The *EcoRI* fragment containing the 3.5-kb β_1 -globin gene from pXG β_1 was then inserted into the unique *EcoRI* site of pJYM Δ RB to produce pJYM β_1 . The open box represents SV40-derived sequences. The thick line represents *X. laevis*-derived sequences. The thin line represents plasmid sequences. Arrows indicate the direction of e (early), l (late) and β_1 (globin) transcription. Approximate locations of DNase I hypersensitive sites A, B and C (Fig. 3) are arrowed. P is the 5' end-labelled *Bcl*I/*Rsa*I *S*₁ mapping probe. Q is the *Bgl*II/*Nco*I probe used for hypersensitive site mapping. ORI marks the approximate location of the SV40 origin.

nuclease mapping with the following results. When introduced alone (pXG β_1 ; Fig. 1 legend), the *X. laevis* β_1 -globin gene was not expressed (Fig. 2a, lane 6). Expression was not rescued by using DNA templates prepared from strains of *Escherichia coli* deficient in methylation (Fig. 2a, lane 7). However, when the gene was inserted into plasmid pJYM Δ RB (Fig. 1 legend), containing SV40 sequences, the resultant recombinant, pJYM β_1 (Fig. 1), produced high levels of β_1 -globin gene transcription (Fig. 2a, lane 5). The pattern of transcription obtained from pJYM β_1 was identical to that seen when RNA isolated from *X. laevis* erythroblasts was mapped using *S*₁ nuclease (Fig. 2a, lane 2). Furthermore, globin-specific RNA isolated from pJYM β_1 -transfected HeLa cells co-migrated on Northern blots with β_1 -globin messenger from *Xenopus* erythrocytes. Therefore, like the rabbit β -globin gene transcripts¹⁷, transcripts from the *X. laevis* β_1 -globin gene are correctly processed in HeLa cells.

Transcription could not be restored by co-transfecting pJYM Δ RB and pXG β_1 (Fig. 2b, lane 3), thereby demonstrating that it is not a result of the action in *trans* of SV40 early gene products. HeLa cells are semi-permissive for SV40¹⁹, and thus allow large-T-antigen-dependent replication of DNAs containing functional SV40 origins. Both pJYM Δ RB and pJYM β_1 produce large-T antigen as judged by immunofluorescence (data not shown), and have intact SV40 origins, thereby allowing them to replicate. pXG β_1 , on the other hand, is devoid of both the SV40 origin and early region. Although we have not formally excluded the possibility that transcriptional rescue is a result of replication, other workers¹⁷ have demonstrated that the enhancer-mediated potentiation of gene transcription is independent of this effect. We therefore consider it likely that the enhancer element in pJYM β_1 is involved in stimulating the expression of the *X. laevis* β_1 -globin gene.

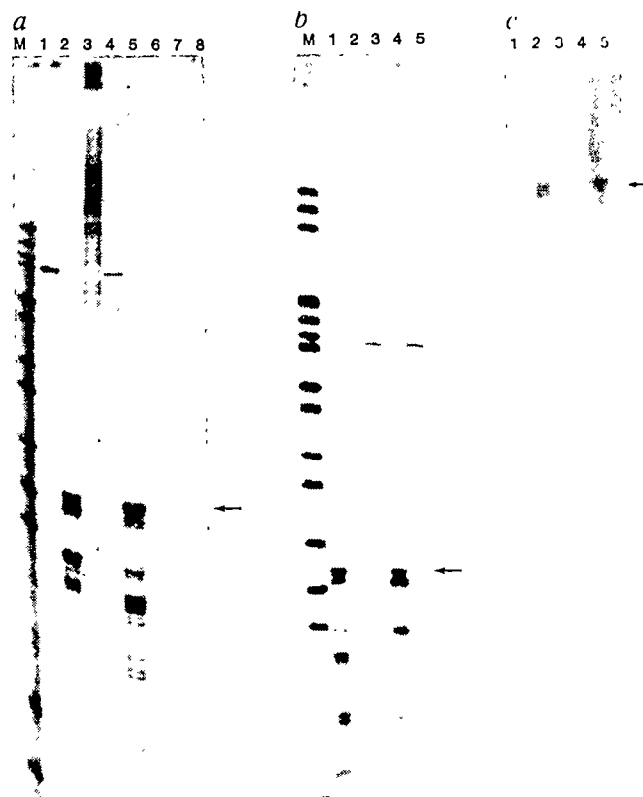


Fig. 2 Analysis of β_1 -globin gene expression in HeLa cells. Total cytoplasmic RNA isolated from HeLa cells 48 h after transfection was analysed for *X. laevis* globin-specific transcription by either *S*₁ nuclease mapping^{31,32}, followed by electrophoresis on denaturing polyacrylamide gels³³ (a, b), or Northern blotting (c). The size marker, M in a and b, is a 3'-end-labelled pAT153 *Hpa*II digest yielding fragment sizes of: 634 (faint), 494 (faint), 407 (faint), 244, 240, 219, 205, 192, 162 (doublet), 149, 124, 112, 92, 78, 69, 36 and 28 base pairs (bp). a, Lane 1, 190-bp *Bcl*I/*Rsa*I fragment used as *S*₁ probe (P in Fig. 1). Lane 2, 225 ng of total cytoplasmic RNA from X.1. erythroblasts. The arrow indicates the protected fragment size for authentic globin mRNA. Lane 3, a Maxam and Gilbert sequencing reaction size marker. Lane 4, pJYM Δ RB. Lane 5, pJYM β_1 . Lane 6, pXG β_1 . Lane 7, pXG β_1 grown in a *dam*⁻ strain of *E. coli*. Lane 8, 5 μ g of transfer RNA. b, Lane 1, pJYM β_1 . Lane 2, pXG β_1 . Lane 3, pJYM Δ RB + pXG β_1 . Lane 4, 225 ng of total cytoplasmic RNA from X.1. erythroblasts. Lane 5, 5 μ g of transfer RNA. The arrow indicates the protected fragment size for authentic β -globin mRNA. c, Lane 1, pJYM Δ RB. Lane 2, pJYM β_1 . Lane 3, pXG β_1 . Lane 4, pXG β_1 *dam*⁻. Lane 5, 225 ng of total cytoplasmic RNA from X.1. erythroblasts. The arrow indicates the size of processed *X. laevis* β_1 -globin mRNA. **Methods.** Transfection and RNA preparation: HeLa cells were transfected by the DEAE-dextran procedure essentially as described elsewhere³⁵ with the following modifications: (1) after removal of the DNA/DEAE-dextran mixture, cells were 'hocked' with 5 ml of phosphate-buffered saline containing 1% glycerol for 1 min; and (2) cells were not treated with chloroquine diphosphate after transfection. After 48 h, the cells were lysed as described in Fig. 3 legend, and total cytoplasmic RNA was prepared as described elsewhere³⁶ and run on neutral agarose gels. Ribosomal RNA profiles were used to evaluate RNA concentration and integrity. *S*₁ nuclease analysis: The *Bcl*I/*Rsa*I fragment (P in Fig. 1) of the *X. laevis* β_1 -globin gene was 5'-end-labelled with ³²P to a specific activity of 1×10^7 c.p.m. per pmol of 5' ends, strand separated on denaturing polyacrylamide gels and electroeluted. Cytoplasmic RNA (15 μ g) isolated from transfected HeLa cells was hybridized to 2×10^5 c.p.m. of probe (probe excess) as described elsewhere³⁷, and digested with *S*₁ nuclease as described previously³² except that the concentration of Zn²⁺ was increased by a factor of 10 to compensate for the high level of EDTA in the hybridization buffer. Northern analysis: Cytoplasmic RNA (15 μ g) isolated from transfected HeLa cells was electrophoresed on a formaldehyde/agarose gel, transferred to nitrocellulose³⁸ and hybridized with a nick-translated³⁹ *Xenopus* β_1 -globin cDNA clone pXG8D2⁴⁰ as described previously¹⁴.

As DNA transfected into cultured cells has been shown to form chromatin²⁰, we compared the chromatin structure of the transcriptionally active globin gene in pJYM β_1 with that of the quiescent copy in pXG β_1 . Given the close correlation between DNase I hypersensitivity and transcriptional activity¹, we chose DNase I as a structural probe. Nuclei from transfected cells were treated with differing concentrations of DNase I, after which total DNA was prepared and analysed by indirect end labelling²¹. This analysis revealed three hypersensitive sites in pJYM β_1 , designated A, B and C (Fig. 3). Site A maps to the late side of the SV40 control region and probably corresponds to the reported DNase I hypersensitivity of the enhancer element itself^{14,20}. Site B occurs in the SV40 late coding region between the initiation codons for viral capsid proteins VP2 and VP3. This site has not been described previously, although the data of Jongstra *et al.*²² reveal a hypersensitive band in the same region. Site C occurs in the 5' region of the *X. laevis* β_1 -globin gene extending roughly from -100 to +50 relative to the start of transcription at +1. This agrees quite closely with the position of the hypersensitive site associated with the *X. laevis* β_1 -globin gene in *Xenopus* erythrocytes (ref. 23 and J. Allan, unpublished). None of these sites is observed when naked DNAs are subjected to DNase I (M. E. Walmsley *et al.*, in preparation).

Note that when no DNase I was added, pJYM β_1 was specifically cleaved by an endogenous nuclease (Fig. 3, lane 6). It can be seen that the three DNase I hypersensitive sites form only a small subset of the regions recognized by the endogenous nuclease. The endogenous cleavage pattern may reflect some other feature of chromatin structure; alternatively, these bands may be the products of cleavage of naked DNA molecules with a marked sequence specificity, a possibility now being investigated in our laboratory. DNase I analysis of nuclei from HeLa cells transfected with pXG β_1 revealed no hypersensitive sites (Fig. 3, lanes 1-5); this was not due to a failure of DNase I, whose activity was confirmed by the reduction in size of HeLa cell genomic DNA as judged by ethidium bromide staining (data not shown).

The concomitant rescue of *Xenopus* β_1 -globin transcription and the induction of hypersensitivity seen in pJYM β_1 was also achieved in another construct, pJYM $\beta_1\Delta 1p$ (Fig. 4). In this construct the late promoter region of SV40 has been deleted, excluding the possibility that the results obtained with pJYM β_1 were due to activation of the globin unit by 'readthrough' late transcription. Furthermore, the orientation of the *Xenopus* β_1 -globin gene relative to the viral early control region has been changed in pJYM $\beta_1\Delta 1p$, reinforcing the long-range nature of the process(es) involved. This could be taken as evidence for a causative role for the SV40 enhancer, which is known to act essentially independently of orientation and distance. However, like pJYM β_1 , pJYM $\beta_1\Delta 1p$ can replicate in HeLa cells. We have not excluded a possible role for replication, whose effects are also relatively position independent, in the activation of the *Xenopus* β_1 gene.

The results presented here reinforce the already strong correlation between DNase I hypersensitivity and transcriptional activity¹. Their novelty resides in the fact that a DNase I hypersensitive site is established by changing the sequence environment of a gene without any concomitant alteration in the complement of cellular *trans*-acting factors. It is unlikely that DNase I hypersensitivity is merely a consequence of transcription, for in a chicken erythroid cell line, DNase I hypersensitivity of the β -globin gene precedes its transcriptional activation²⁴. Our results are particularly pertinent given the association between the binding of *trans*-acting factors and hypersensitivity¹⁵, together with the demonstration that the reconstitution *in vitro* of the chicken globin gene DNase I hypersensitive site is dependent on development- and tissue-specific factors^{2,3}. Weintraub, however, has proposed¹⁶ that the nuclease-hypersensitive 5' and 3' regulatory regions of eukaryotic genes have the potential to form an altered DNA secondary structure, and that this ability is contained within the DNA sequence alone. He demonstrated

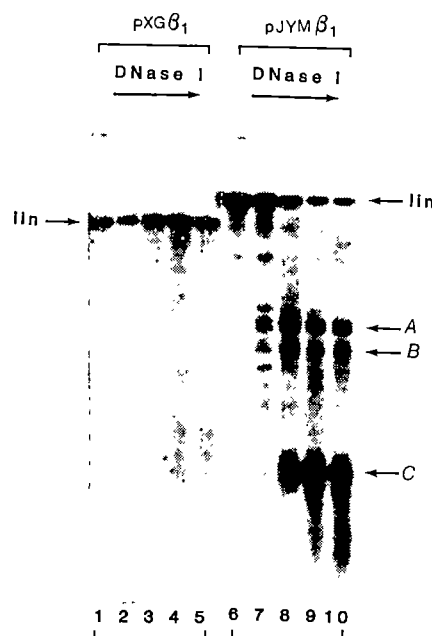


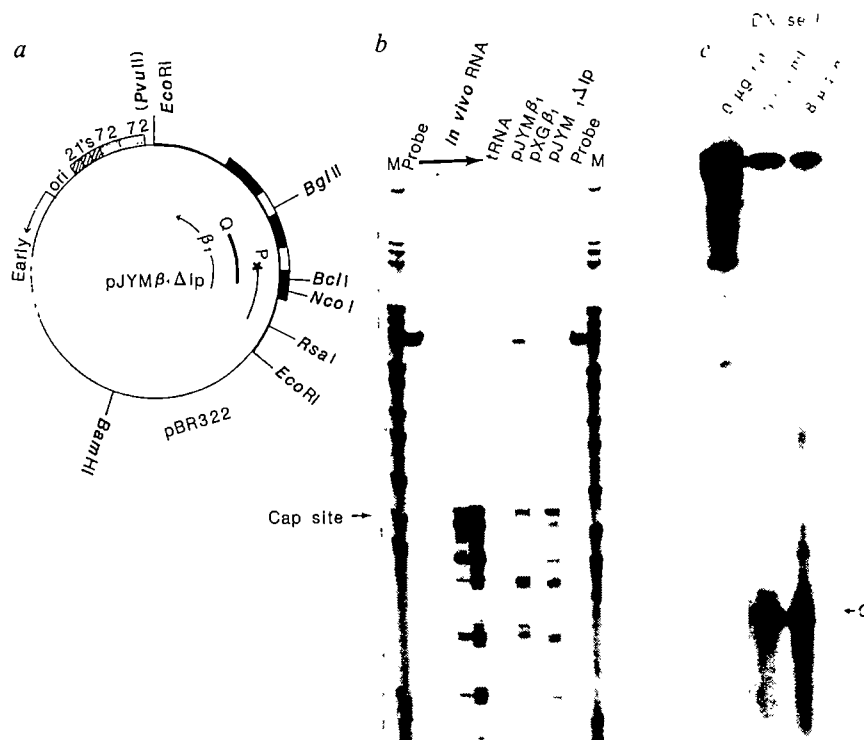
Fig. 3 Indirect end-labelling analysis of DNase I-treated transfected material. Nuclei were prepared from transfected HeLa cells after 48 h and treated with DNase I. Total DNA was then restricted with *Bgl*II and hybridized with a *Bgl*II/*Nco*I probe (Q in Fig. 1). The linear main band is marked 'lin' and hypersensitive sub-bands are arrowed A, B and C. Lanes 1-5 represent nuclei from cells transfected with pXG β_1 treated with 0, 1, 2, 5 and 8 $\mu\text{g ml}^{-1}$ DNase I (Worthington) respectively. Lanes 6-10 represent the same concentration course performed on nuclei from cells transfected with pJYM β_1 .

Methods. Trypsinized HeLa cells from a confluent 250-ml flask were washed once in RPMI 1640 medium containing 20 mM HEPES, resuspended in 0.72 ml of ice-cold lysis mix (50% glycerol, 50 mM Tris 7.9, 100 mM KCl, 5 mM MgCl₂, 0.05% saponin and 200 mM β -mercaptoethanol) and left on ice for 10 min. The lysed mixture was spun at 5,500 r.p.m. for 15 min at -5°C in a Sorvall HB4 fitted with Eppendorf tube adaptors. The supernatant was removed for RNA preparation and the nuclear pellet was resuspended in 0.8 ml of ice-cold buffer A (100 mM NaCl, 50 mM Tris pH 8.0, 3 mM MgCl₂, 0.1 mM phenylmethylsulphonyl fluoride), then repelleted in the same rotor assembly for 5 min at 3,000 r.p.m., 0°C . The nuclear pellet was then resuspended at 500 $\mu\text{g ml}^{-1}$ in ice-cold buffer A. To 18- μl aliquots of this was added 2 μl of differing dilutions of DNase I in buffer A; these reactions were incubated for 30 min at 37°C before termination by addition of 1 μl of 0.5 M EDTA pH 8.0. RNase was added to a final concentration of 20 $\mu\text{g ml}^{-1}$ and after a further 30-min incubation at 37°C , proteinase K and SDS were added to 50 $\mu\text{g ml}^{-1}$ and 0.25%, respectively, and the samples left overnight at 37°C . Sample volumes were increased to 100 μl before extraction with phenol/chloroform and chloroform, followed by ethanol precipitation. Resuspended DNAs were restricted overnight with *Bgl*II, electrophoresed on 0.8% agarose gels, transferred to nitrocellulose⁴¹ and hybridized with a ^{32}P -labelled *Bgl*II/*Nco*I fragment (Q in Fig. 1) prepared as described elsewhere⁴².

that these secondary structures observed *in vivo* may be elicited *in vitro* by DNA supercoiling.

We speculate that our observations are best interpreted by a mechanism involving both *cis*- and *trans*-acting components, and envisage a scenario whereby the SV40 early control region binds a *trans*-acting factor(s) which in turn direct(s) the binding of a DNA gyrase-like activity that induces DNA supercoiling. DNA sequences in the proximity of the globin hypersensitive site, with a propensity to form altered secondary structures under superhelical stress, adopt conformations which directly or indirectly discourage the placement of nucleosomes at the hypersensitive site. Supercoiling has already been implicated in generalized DNase I sensitivity²⁵ as well as transcriptional activity²⁶. This, together with the recent observation that the SV40 enhancer region is the major site of action of drug-induced cleavage by

Fig. 4 Analysis of a SV40-*Xenopus* recombinant genome lacking the viral late region. **a**, Map of pJYM $\beta_1\Delta 1p$. This construct is identical to pJYM β_1 except that the *EcoRI*/*PvuII* fragment containing viral late sequences has been removed, and the *Xenopus* globin gene fragment has been inverted. **b**, S_1 nuclease analysis of RNA isolated from HeLa cells transfected with pJYM $\beta_1\Delta 1p$. Probes, markers and methods as for Fig. 2. The *in vivo* RNA lanes contain 1.0, 10, 100 and 1,000 ng of total cytoplasmic RNA isolated from *X. laevis* erythroblasts. **c**, DNase I analysis of nuclei isolated from HeLa cells transfected with pJYM $\beta_1\Delta 1p$. Methods as for Fig. 3. The hypersensitive site of the *Xenopus* β_1 -globin gene is marked C.



mammalian DNA topoisomerase II²⁷, lends credence to this model. Whatever the mechanism of the induction, our data clearly demonstrate that hypersensitive sites can be induced *in vivo*.

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Assembly of microtubules from nucleotide-depleted tubulin

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In vitro assembly of microtubules from tubulin is considered to have an absolute requirement for added GTP (or a non-hydrolysable GTP-analogue)¹ involving binding at the E(exchangeable)-site located on the β -subunit of the tubulin dimer². By contrast, GDP inhibits assembly. Nucleotide hydrolysis has been implicated in the dynamic properties of microtubules^{3,4}, treadmilling⁵ and mechanical coupling⁶. Here we demonstrate that assembly is not necessarily dependent on the presence of GTP at the E-site; microtubules can be formed efficiently in the absence of GTP in the presence of pyrophosphate. These microtubules, which have normal morphology and lability at cold temperatures, contain N(non-exchangeable)-site GTP and a significant proportion of E-site GDP. This demonstrates the possibility of direct incorporation of GDP-containing tubulin dimer during assembly which probably derives from microtubule-associated protein (MAP)-containing oligomers. This finding has important implications for the mechanism of microtubule elongation. The effects of pyrophosphate suggest that charge neutralization by the bidentate ligand is an essential step in promoting microtubule assembly, and that this interaction involves only a minimal conformational change in the protein.

Studies of nucleotide-depleted tubulin are restricted by high nucleotide affinities⁷ and protein instability⁸⁻¹⁰. We have quantitated the removal of E-site GDP by alkaline phosphatase¹¹, using HPLC to follow nucleotide hydrolysis. The progressive loss of GDP (at 20 °C) is faster and more extensive from tubulin dimer than from microtubule protein (tubulin + MAPs). After treatment for 25 min, the assembly capability of this microtubule

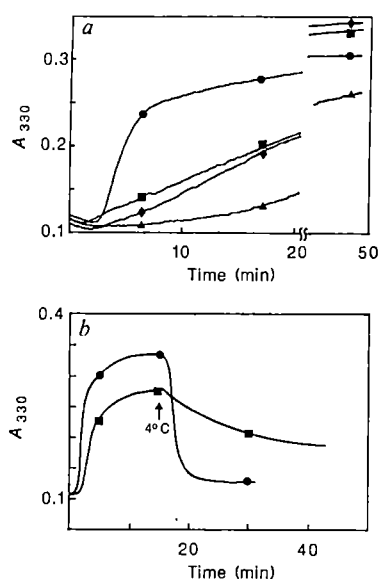


Fig. 1 Pyrophosphate-induced assembly of nucleotide-depleted microtubule protein at 37 °C in the absence of GTP. *a*, Absorbance (330 nm) of microtubule protein (1.2 mg ml⁻¹) treated with alkaline phosphatase for various times at 20 °C, before addition of 3 mM pyrophosphate and incubation at 37 °C in the absence of GTP. The protein was incubated with alkaline phosphatase for 0 (▲), 10 (■) and 25 (●) min. Control (◆), no fast assembly is observed after treatment with alkaline phosphatase for 25 min in the absence of pyrophosphate. *b*, Assembly (37 °C) and disassembly (4 °C) of microtubule protein in 1 mM GTP (●) and of alkaline phosphatase-treated microtubule protein in 3 mM pyrophosphate (■). Protein concentration, 1.2 mg ml⁻¹.

protein (on re-addition of 1 mM GTP, plus 3 mM pyrophosphate to inhibit further alkaline phosphatase action) is >90%, showing that nucleotide removal is reversible.

If pyrophosphate is added after alkaline phosphatase treatment (but without subsequent addition of GTP) a specific pyrophosphate-induced microtubule assembly is observed (Fig. 1). Pyrophosphate added to the nucleotide-depleted microtubule protein (containing 40% E-site GDP) causes a rapid increase in turbidity at 37 °C (Fig. 1*a*, circles), superimposed on the more gradual background increase. The rapid process is caused by microtubule formation (see below); it does not occur if the pyrophosphate is either omitted or added for shorter periods (Fig. 1*a*). Neither phosphate, sulphate nor triphosphate can substitute for pyrophosphate. There is maximal effect at 3–5 mM pyrophosphate, whereas higher concentrations of pyrophosphate are inhibitory. Although prior addition of 1 mM GDP inhibits the rapid effect, there is little effect with addition at plateau: the pyrophosphate-induced assembly does show partial cold lability (Fig. 1*b*).

To eliminate potential effects of dephosphorylation of MAPs by alkaline phosphatase, nucleotide-depleted tubulin dimer was prepared from phosphocellulose-purified tubulin by treatment with alkaline phosphatase for 15 min; the resultant tubulin contained ~20% E-site GDP. Following addition of heat-treated intact MAPs¹² (not exposed to alkaline phosphatase), the nucleotide-depleted tubulin dimer assembled at 37 °C with 3 mM pyrophosphate.

Electron microscopy shows microtubules of normal morphology in the pyrophosphate-induced assembly. The microtubules show well-defined protofilament structure and the rough-walled appearance characteristic of the presence of MAPs (Fig. 2*a*). These microtubules are distributed uniformly throughout the samples (Fig. 2*b*). After disassembly at cold temperatures only amorphous material is seen. When alkaline phosphatase-treated microtubule protein is incubated at 37 °C without pyrophosphate, similar amorphous material is produced (Fig. 2*c*). The microtubules are relatively short compared with those assembled with microtubule protein plus GTP. Micro-

tubules of normal morphology are also formed from nucleotide-depleted tubulin dimer with MAPs as described above (Fig. 2*d*).

The nucleotide content of the pyrophosphate-induced microtubules was analysed by HPLC (Table 1). The initial Sephadex G-25 treated protein contains 1/1 molar ratio GTP/GDP; the E-site nucleotide is >97% GDP. The GTP content indicates tightly bound nucleotide at the N-site, which serves as a marker of the proportion of tubulin retaining structural integrity. Alkaline phosphatase removes GDP from the E-site. On resuspension of the 37 °C microtubule pellet at 0 °C, 86% of the nucleotide-containing tubulin appears in solution, and thus derives from cold-disassembled nucleotide-depleted microtubules. From the GDP/GTP ratio, these microtubules contain nucleotide in the proportion 0.5 mol E-site GDP per mol N-site GTP. Using ³H-GDP, this GDP is shown to derive entirely from E-site GDP bound to tubulin before assembly.

Our results show that nucleotide-depleted microtubule protein can be assembled in the absence of GTP into morphologically

Table 1 Nucleotide content of pyrophosphate-induced microtubules assembled at 37 °C from nucleotide-depleted microtubule protein in the absence of added GTP

	GTP	GDP	GMP/Guo	Total
Microtubule protein (Sephadex G-25 eluate):	100	94	4	(198)
After alkaline phosphatase treatment and assembly at 37 °C:				
S1	86	38	75	(199)
P1	52	20	78	
P1	29	14	3	(196)
0 °C resuspension of P1:				
S2	25	12	0	
P2	0	0	0	
Ratio S2/P1 (%)	86	86		

Alkaline phosphatase (40 U ml⁻¹) was added to 2.0 mg ml⁻¹ Sephadex G-25-treated bovine microtubule protein¹⁵ (25 min at 20 °C), followed by addition of 3 mM pyrophosphate. After incubation at 37 °C for 15 min, centrifugation (for 5 min at 140,000g in the Airfuge; A100 rotor) of the sample gave a supernatant (S1) and pellet (P1). After resuspension of P1 at 0 °C for 30 min in MEM100 buffer¹⁵, centrifugation gave supernatant S2 and pellet P2. The nucleotide content (mean data of seven experiments), was assessed by HPLC and is expressed relative to initial N-site GTP as 100%; bracketed figures indicate total nucleotide recovery at different stages. Electrophoresis and densitometry of Coomassie blue-stained SDS-polyacrylamide 7.5% gels shows that 47% total protein is pelleted in P1; this material shows a normal ratio of MAPs to tubulin (16% HMW1 and 2). Guo = guanosine.

normal, cold-labile microtubules. There is no evidence that contaminating GTP is responsible for the assembly. We observe short microtubules, indicating efficient nucleation; pyrophosphate is implicated in both the nucleation and elongation processes.

It is striking that the pyrophosphate-induced assembly of nucleotide-depleted microtubule protein gives microtubules which contain substantial proportions of tubulin-GDP. The mechanism of tubulin-GDP incorporation during elongation of microtubules is controversial^{1,13}. Isotope labelling¹⁴, assembly kinetics¹⁵ and time-resolved X-ray scattering¹⁶ techniques indicate direct incorporation of oligomeric fragments into assembling microtubules. The E-site GTP/GDP content of assembling oligomeric fragments is not well-defined. We have shown, however, that in nucleotide-depleted microtubule protein the GDP-tubulin is entirely associated with MAP-containing oligomers. Our present work therefore raises the possibility that fragments containing substantial proportions of tubulin-GDP could be incorporated as such, that is, without prior conversion to tubulin-GTP. We have recently demonstrated¹⁷ that this occurs for the assembly of microtubule protein induced by the GTP analogue GMPPCP. This indicates a minimum requirement for GTP (or an analogue such as GMPPCP or pyrophosphate), possibly at the elongation site, but not necessarily within the

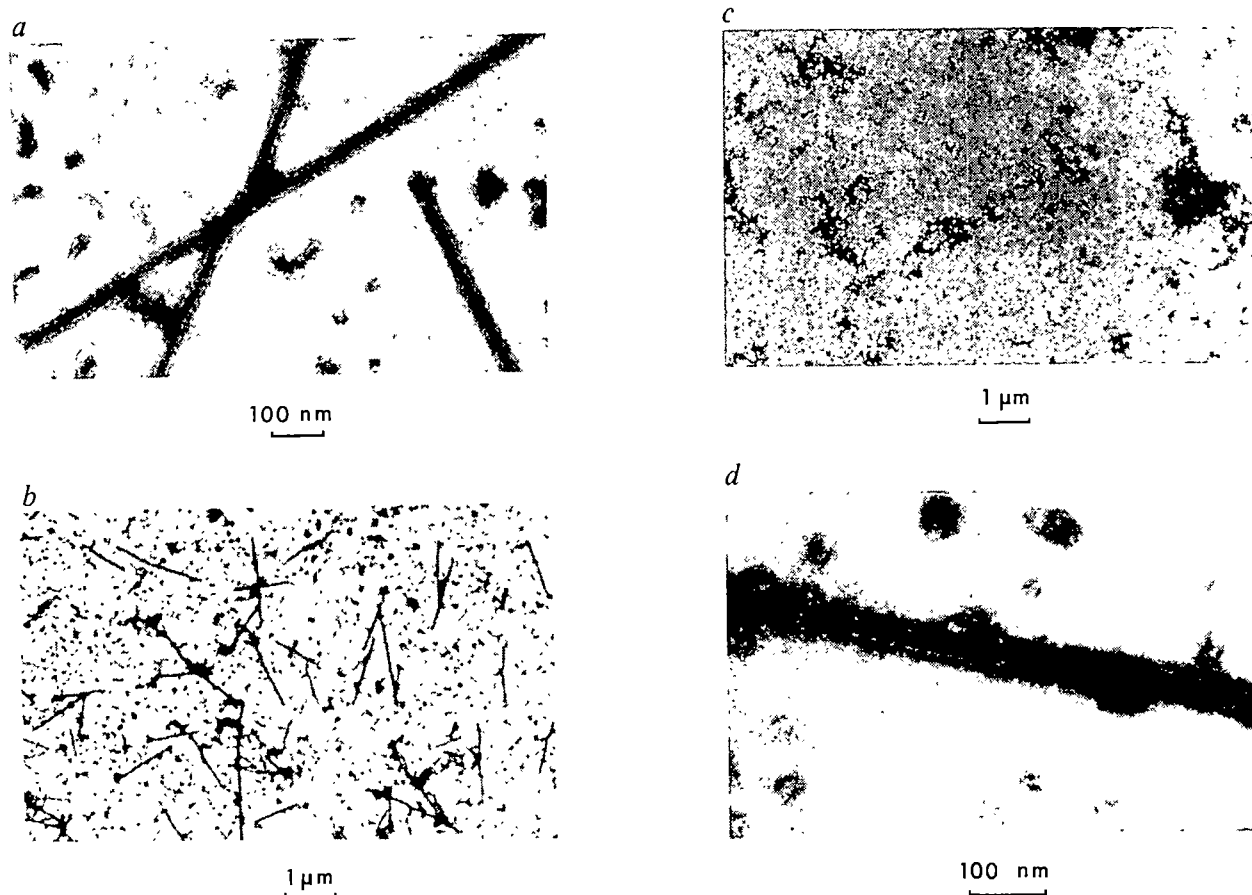


Fig. 2 Electron micrographs of products of assembly with nucleotide-depleted microtubule protein or tubulin plus MAPs at 37 °C with pyrophosphate. Nucleotide-depleted protein was prepared by treatment with alkaline phosphatase at 20 °C as described in Fig. 1 legend; microtubule protein (*a-c*) was incubated for 25 min, tubulin dimer (*d*) for 15 min. *a*, Addition of 3 mM pyrophosphate before 37 °C incubation; *b*, low-magnification view of *a*; *c*, control 37 °C, no pyrophosphate; *d*, nucleotide-depleted tubulin dimer plus normal MAPs (see text) incubated at 37 °C with 3 mM pyrophosphate. (Samples *a* to *d* were prepared for electron microscopy by negative staining as described in ref. 15.)

oligomeric fragment itself. Thus, the nucleotide requirements for assembly of microtubule protein appear to be less stringent than those for pure tubulin dimer.

Direct interaction of pyrophosphate with tubulin has been shown by competition experiments with ^3H -GDP (unpublished data). As a mechanism for pyrophosphate-induced assembly, we propose that this ion binds to tubulin at the E-site in locations corresponding to the high-affinity sites for β - γ positions of GTP. The pyrophosphate ion promotes assembly of nucleotide-depleted tubulin without inducing major secondary or tertiary structural changes^{18,19}. The adoption of an assembly-competent state of tubulin could therefore require no more than the simultaneous occupancy of two potentially adjacent positively charged sites by an appropriate bidentate negatively charged ligand, of which pyrophosphate is a simple example. We suggest that by such an alignment of two protein domains, a minimal protein conformational change facilitates the required interactions for polymerization.

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The CO bond angle of carboxymyoglobin determined by angular-resolved XANES spectroscopy

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Our knowledge of the structure of condensed matter has been based primarily on spectroscopic methods that measure first-order pair correlations of atomic arrangements and thus provide interatomic distances (for example neutron and X-ray scattering). Bond angles are given by higher-order correlation functions, and such information can be provided by X-ray absorption near-edge structure (XANES) spectroscopy^{1,2}, the features of which are determined by multiple scattering of photoelectrons whose paths

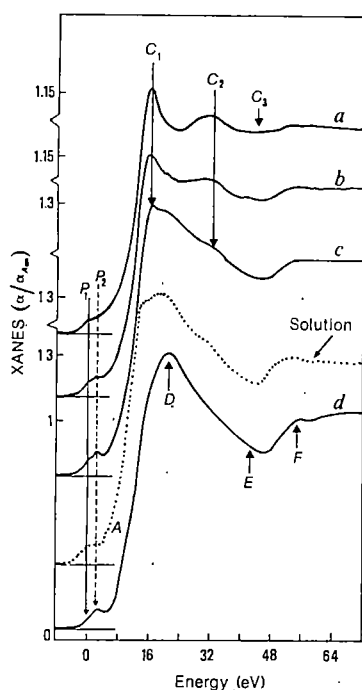


Fig. 1 Experimental XANES spectra (a-d) of a single MbCO crystal for different orientations of the crystal with respect to the polarization vector \vec{E} . The crystal was rotated from the $\vec{E} // \hat{a}^*$ (curve a) to the $\vec{E} // \hat{c}$ (curve d) orientation. The calculated angles φ between \vec{E} and the haem normal \hat{n} are: a, 28°; b, 41°; c, 62°; d, 85°. The experimental XANES spectrum of MbCO in solution is shown dotted.

begin and end at the selected absorbing atom. We report here angular-resolved XANES spectroscopy of a single crystal of carboxymyoglobin (MbCO). The large dichroism of the X-ray absorption of the crystal can be fully interpreted by multiple-scattering theory which allows the determination of Fe-ligand bond angles. The analysis of the identified multiple scattering features due to CO in high signal-to-noise-ratio spectra of protein in solution has allowed the determination of the variation of CO bond angles. This opens the way to the determination of subtle structural features due to bond angle variations in proteins in solution which are relevant to an understanding of the characteristics of proteins at the atomic scale.

Knowledge of Fe-C-O and Fe-O₂ bond angles in solution is fundamental to the understanding of reversible molecular binding of diatomic molecules in haem proteins, but current data are limited to results obtained by crystallography. In fact, only infrared CO stretching and ¹³C nuclear magnetic resonance (NMR) spectroscopy are available to probe CO bonding in proteins in solution; moreover, many factors affect the spectroscopic signals and no simple interpretation has been established in terms of protein structure^{3,4}. XANES spectroscopy is useful in that it is a direct structural probe which can be used to study both crystals and solutions.

We first applied XANES spectroscopy to a MbCO single crystal, a system which has been extensively studied by crystallography. The photoelectron multiple scattering resonances in the direction of CO have been identified which probe the three-atom correlation function and therefore give the C-O bond angle. The Fe-C-O configuration has been found to be bent, with a Fe-C-O bond angle of ~150°; this agrees qualitatively with neutron diffraction data⁵, although that technique is subject to large errors.

We then used the XANES method to study the Fe-C-O bond angle in haem proteins in solution. The infrared CO stretching signal in MbCO changes on going from crystal to solution⁶, and this has led to the suggestion that the FeCO configuration is different in solution. On the other hand, La Mar *et al.*⁷ found no difference between the ¹³C-NMR signal of MbCO and the

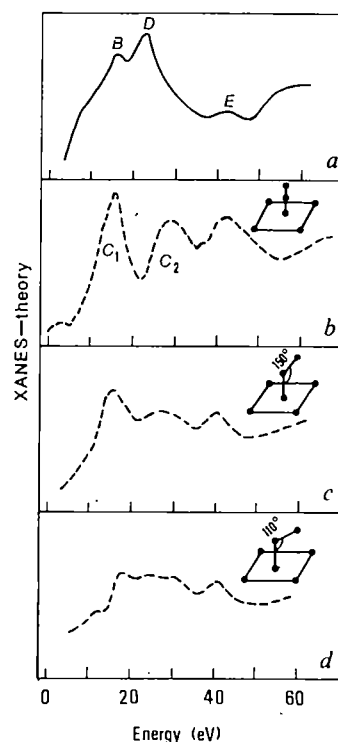


Fig. 2 Theoretical angular resolved XANES. a, The experimental features A, B, D, E and F due to the scattering in the haem plane, $\vec{E} \perp \hat{n}$, are predicted by the theory. b-d, The $\vec{E} // \hat{n}$ XANES curves for various CO bond angles. The best agreement between the theoretical and experimental spectra was found for the Fe-C-O configuration at $\theta = 150^\circ$.

unhindered synthetic haem model, and suggested that there was no distortion of the FeCO configuration in MbCO in solution. This is in contrast to the bent configuration observed in crystals. We have found that MbCO in solution shows a Fe-C-O configuration that is bent at $\theta \approx 150^\circ$, as occurs in the crystal. However, for carboxyhaemoglobin (HbCO) in solution we found $\theta = 165^\circ$ whereas crystallographic studies of HbCO crystals found a smaller value of $\theta = 136^\circ$ (ref. 8).

A large ($5 \times 3 \times 0.8$ mm) MbCO single crystal (space group P2₁) was oriented in a collimated X-ray synchrotron radiation beam completely intercepted by the crystal. The XANES spectra were measured by recording the intensity of the FeK α fluorescence as a function of the X-ray photon energy, and the crystal was rotated in steps around the *b*-axis from the $\vec{E} // \hat{a}^*$ to the $\vec{E} // \hat{c}$ orientation, where \hat{a} and \hat{c} are the crystallographic axes and \vec{E} is the polarization vector. The data on the crystals were recorded using synchrotron radiation sources at Daresbury Laboratory and those on the 5 mM HbCO, MbCO and MbCN solutions were recorded at Frascati Laboratory.

Figure 1 shows the angular resolved FeK XANES of the MbCO single crystal. The polarized X-ray absorption spectra can be classified according to the angle φ between \vec{E} and the haem normal axis \hat{n} . The MbCO spectrum for $\vec{E} // \hat{a}^*$ ($\varphi = 28^\circ$) is mostly due to multiple scattering along the haem normal and for $\vec{E} // \hat{c}$ ($\varphi \sim 86^\circ$) the spectrum is mostly due to photoelectron multiple scattering in the haem plane. Features C₁ and C₂ clearly arise from photoelectron multiple scattering along the normal direction, while features A, B, D, E and F arise from scattering in the haem plane. All are present in the spectrum of MbCO in solution (Fig. 1).

The theoretical FeK XANES of MbCO was calculated using the full multiple scattering approach (refs 2, 9-11 and S. Hasnain, A.B. and S.P., unpublished), in which the absorption cross-section for one-electron excitations from a core level is determined by the sum of all scattering paths for the photoelectron which begin and end on the same absorbing Fe atom at the origin. Figure 2 shows several theoretical angular-resolved XANES spectra of MbCO. The atomic coordinates of the cluster

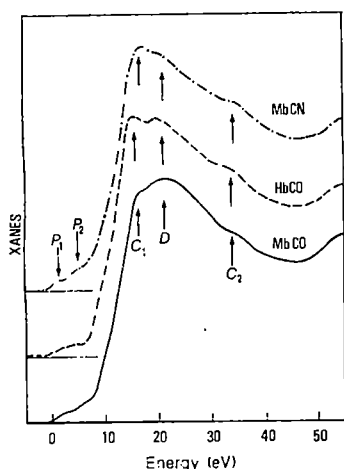


Fig. 3 Experimental XANES spectra of MbCO, HbCO and MbCN in solution. The main difference is the variation of the intensity ratio between peak C_1 and peak D which depends on the bonding angle Fe-C-O(N).

of 30 atoms have been deduced from diffraction data⁵ and polarized extended X-ray absorption fine structure (Fe-N_P = 2.0 Å, F-N_e = 2.09 Å, Fe-C = 1.84 Å) (S. Hasnain, A.B. and S.P., unpublished), and an idealized symmetrical and plane porphyrin ring was used. Figure 2a shows that the theory predicts all the experimental features A, B, D, E, F due to the scattering in the haem plane, while Fig. 2b-d shows the large variation of the \tilde{E}/\hbar spectra obtained by changing the CO bonding geometry. The best agreement between the theoretical spectrum and the experimental results was found for the Fe-C-O bent configuration at $\theta = 150^\circ$. Using the crystallographic coordinates, which have large errors⁵, with Fe-C at 1.78 Å and C-O parallel but displaced from the haem normal axis, we obtain a large disagreement with the XANES experiment.

Finally, having demonstrated the XANES method for a single crystal, we next applied it to determine the bonding geometry of ligands in haem proteins in solution, where diffraction methods cannot be applied. Figure 3 shows the Fe XANES spectra of MbCO, HbCO and MbCN in solution with good signal-to-noise ratio. The main difference between these spectra is the variation of the intensity ratio between peaks C and D , which depends strongly on the Fe-C-O(N) bonding angle. Both by calculation and by comparison with crystal XANES spectra, we find the Fe-C-O angle in solution to be $\sim 150^\circ$, as it is in MbCO crystals.

Theoretical XANES analyses indicate that the Fe-C-O(N) angle is 150° in MbCO, 165° in HbCO and 180° in MbCN in solution. Our results strongly support the Moffat hypothesis¹² that the CN configuration in HbCN is linear and tilted rather than bent. We find, within the sensitivity of the XANES method, that the Fe-C-O configuration is bent in both crystals and solutions, and we estimate that we can easily detect variations of $\Delta\theta = 10^\circ$. We have obtained experimental evidence that the (Fe-C=O) configuration is different in HbCO and MbCO in solution ($\Delta\theta = +15^\circ$ going from MbCO to HbCO) and therefore that the CO bonding is modified by the protein. Crystal diffraction studies have shown a different bent configuration, with $\theta = 136^\circ$ in HbCO (ref. 8). Our data therefore indicate that there is a difference in CO bonding in HbCO in the crystalline and solution phases.

In conclusion, our results show that there is a large dichroism in the X-ray range of the haem protein crystal which can be fully analysed and its bond angles determined quantitatively by XANES. This method can be used to improve our knowledge of the subtle variations that occur in the local structure of proteins and can thus help us to establish the relationship between localized structural changes and the mechanism of protein control of ligand binding in solution close to the protein native state.

We thank M. Perutz for discussions and hospitality at the MRC Laboratory in Cambridge where the MbCO crystal was prepared, and the synchrotron radiation facilities of the SERC Daresbury Laboratory and of the INFN-CNR Frascati Laboratory. This project has been partially supported by the office for international collaboration and GNCB of the Consiglio Nazionale delle Ricerche.

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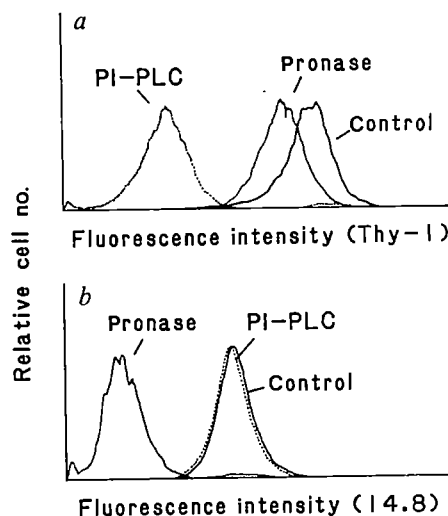
Errata

Phosphatidylinositol is the membrane-anchoring domain of the Thy-1 glycoprotein

M. G. Low & P. W. Kincade

Nature 318, 62-64 (1985)

In Fig. 1a and b, two lines representing the incubation with phosphatidylinositol-specific phospholipase C (PI-PLC) were not properly reproduced. The correct figure is shown here:



More help required on T and B cells

J. Shifflett

Nature 316, 490 (1985)

THE word 'antigen' was omitted from the second sentence in paragraph 2, which should read: 'B lymphocytes might find it difficult to pinocytose a surface antigen of any pathogen unwilling to facilitate its own destruction...' In addition, the second sentence in the third paragraph is made clearer with the words 'of materials' deleted.

Shanghai Institute of Biochemistry: The molecular biology revolution

A. Anderson

Nature 318, 217-218 (1985)

IN this item in the 'Science in China' feature, the deputy-director of the Shanghai Institute of Biochemistry was inadvertently referred to as director. The director is Professor Lin Qi-shui, and his deputy is Professor Zhang You-shang.

Chemistry

Advances in Heterocyclic Chemistry. ALAN R. KATRITZKY (ed.). Academic: 1985. Pp.374. Hbk ISBN 0-12-020638-2. \$87.50, £75.50.

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Dr S. Artavanis-Tsakonas, Crete Workshop, Yale University, Department of Biology, P.O. Box 6666, New Haven, CT 06511-8112, USA.

It is expected that a registration fee of \$200 will be remitted upon acceptance. Local expenses in Crete will be covered. Participants are expected to finance their travel to Crete, however, a small number of grants in aid for partial travel support may become available.

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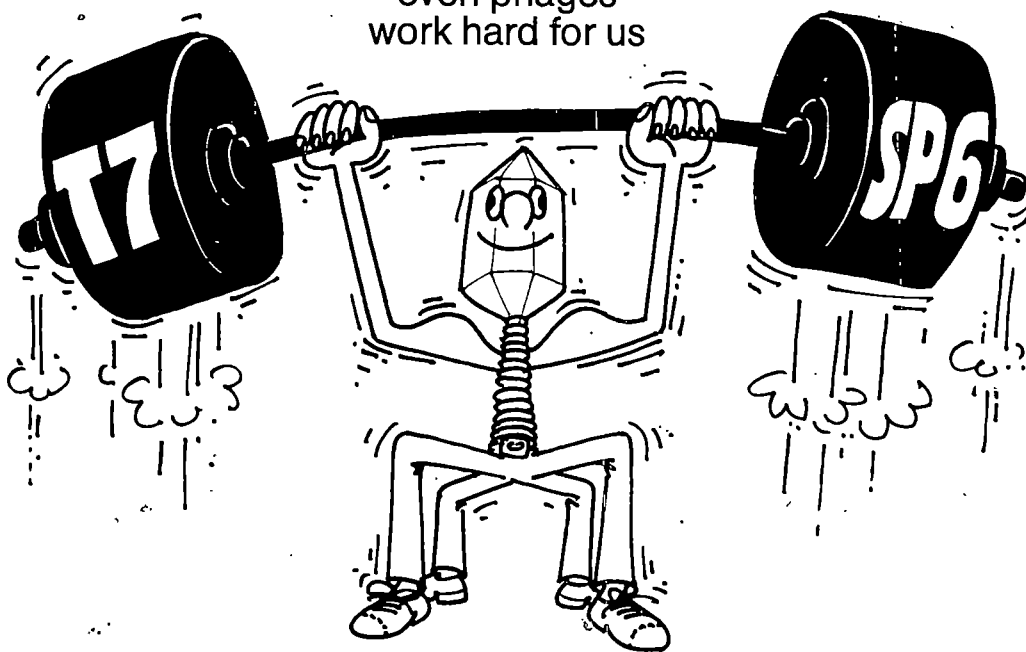
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